THE CHEMICAL STATUS OF BEAN PLANTS AFFLICTED WITH BICARBONATE-INDUCED CHLOROSIS[1]

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Introduction

The waters of many streams used for irrigation in western United States contain a relatively high proportion of bicarbonate in the dissolved solids (**9**). STABLER (**44**) predicted as early as 1911 that use of an irrigation water containing appreciable bicarbonate could lead to the development of a "black alkali" soil when the milliequivalents per liter (m.e./l.) of sodium exceeded the m.e./l. of chloride plus sulfate. EATON (**11, 12**) has recently contributed a significant impetus to the above conclusion from his observations in the Nile and Euphrates valleys. In the water of the Nile the concentration of sodium consistently exceeds that of chloride plus sulfate, whereas the converse situation prevails in the water of the Euphrates. "Black alkali" occurs frequently in the soils of the Nile Valley but is nonexistent in that of the Euphrates. A "black alkali" soil may have one or more of the following attributes: pH value above 8.5, exchangeable-sodium percentage exceeding 15, and abnormally high concentrations of sodium carbonate and bicarbonate in the soil solution. FIREMAN and REEVE (**13**) explained the prevalence of a high content of exchangeable sodium in the calcareous soils of Gem County, Idaho, as a result of the historic flooding of the area by the Payette River in which the dissolved salts are almost entirely sodium and calcium bicarbonate. HARLEY and LINDNER (**20**) observed that application of irrigation waters relatively high in bicarbonate would induce chlorosis of apple and pear trees in Washington and that subsequent irrigation with water low in bicarbonate tended to alleviate the condition. Several workers (**19, 21, 48, 49**) recognized that many of the sources of water for greenhouse crops in Oklahoma contained excessive amounts of salt or "alkali" and carried out studies to ascertain quantitatively the degree of injury of such waters to tomatoes and other greenhouse crops. The presence of added $NaHCO_3$ in water used for growing tomatoes in sand cultures was found to be much more injurious than NaCl (**21**). The high toxicity of water containing $NaHCO_3$ could be largely eliminated by neutralizing the solution to pH 5.6–6.0 with H_2SO_4 (**19**). It was concluded that the high toxicity of $NaHCO_3$ was due to the high pH value (about 8.0–8.5) of the solutions (**49**). Marked differences in the tolerance of different species to $NaHCO_3$ in the irrigation water were observed; for example, carnations were virtually insensitive, whereas chrysanthemums were found to be extremely intolerant, in that they developed intense chlorosis at comparatively low concentrations of $NaHCO_3$(**48**).

GAUCH and WADLEIGH (**17**) also noted

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marked variation in tolerance of different species to $NaHCO_3$ in the culture solution. Rhodes grass was insensitive to the presence of 12 m.e./l. of this salt, whereas this concentration induced such severe chlorosis in Dallis grass as eventually to kill the plants. A control nutrient solution adjusted to pH 8.0 with NaOH but nearly free of bicarbonate did not have this drastic effect on Dallis grass, so these authors concluded that the bicarbonate ion was specifically toxic over and above the effect of high pH values associated with solutions of $NaHCO_3$.

The foregoing observations indicate that the presence of the bicarbonate ion in the substrate may adversely affect growth of certain plants in a unique manner as compared with effects of other ions commonly present. The study reported herein was undertaken to ascertain the influence of bicarbonate in the substrate on the growth and chemical components of a sensitive species. The Dwarf Red Kidney bean (*Phaseolus vulgaris* L.) was selected as a result of preliminary trials showing its marked sensitivity.

Material and methods

The plants were grown in automatically irrigated sand cultures (**14**), with the base nutrient solution containing the following salts per liter: $Ca(NO_3)_2$, 5 m.e.; $MgSO_4$, 3 m.e.; KH_2PO_4, 0.5 m.e.; K_2HPO_4, 0.5 m.e.; KNO_3, 2 m.e.; and K_2SO_4, 2 m.e. One p.p.m. iron was added as ferric citrate; 0.5 p.p.m. boron as H_3BO_3; and 0.5 p.p.m. manganese as $MnSO_4$. Seeds were planted on February 12, 1948, and three seedlings were allowed to grow in each culture. Differential treatment was initiated on February 26. Treatments involved addition of 0, 8, 16, and 32 m.e./l. of $NaHCO_3$ to the nutrient solution of nine cultures at each level. The solutions with no added bicarbonate were adjusted daily to pH 8.0 by the addition of NaOH and were completely changed at least once a week. Solutions with added $NaHCO_3$ had a pH of about 8.0 as made up; when the value went up as high as 8.3, these solutions were renewed, i.e., every few days. Each culture contained three plants.

Plants were harvested from an extra four cultures on the day differential treatment was initiated (February 26, 1948). Harvests of plants from three cultures of each treatment were made 7, 14, and 28 days later. The plants were washed with distilled water from a sprayer on the day before harvesting. At the time of harvest, 20 sq. cm. of tissue were randomly selected from the leaves with a Ganong leaf punch. Blades of primary leaves and trifoliolate leaves were treated separately, and these were differentiated from the stems and petioles. Thus, samples from primary leaves show advancing senescence with successive harvests, whereas samples of trifoliolate leaves include leaves of varying age. The fractions of tissue from each cultural treatment were dried rapidly in a large forced-draft oven maintained at 70°–80° C.

The samples removed by the leaf punch were immediately refrigerated in weighing bottles, and chlorophyll was extracted by JACOBSON'S procedure (**29**) on the day of sampling. Chlorophylls *a* and *b* were determined by the method of COMAR and ZSCHEILE (**10**) using a Beckman spectrophotometer. On harvesting, precautions were taken to avoid contamination of the tissue with iron. The dried material was ground in a porcelain ball-mill using flint pebbles. Total and extractable iron were determined according to OSERKOWSKY'S procedure (**36**) with the exception that O-phenanthroline (**42**)

was used to assay iron colorimetrically.

A weighed portion of the dry tissue was wet-ashed (18), and calcium, sodium, potassium, magnesium, and phosphorus were determined according to the procedures outlined by REITEMEIER (40).

Organic acid content of the dried tissue was determined by the method of PUCHER *et al.* (39).

Observations

GROWTH.—During the first week following the initiation of differential treatment there was little variation in growth response, but thereafter there were marked differences on the various treatments (fig. 1). The "control" plants grew well on the selected culture solution maintained at pH 8.0 but practical-

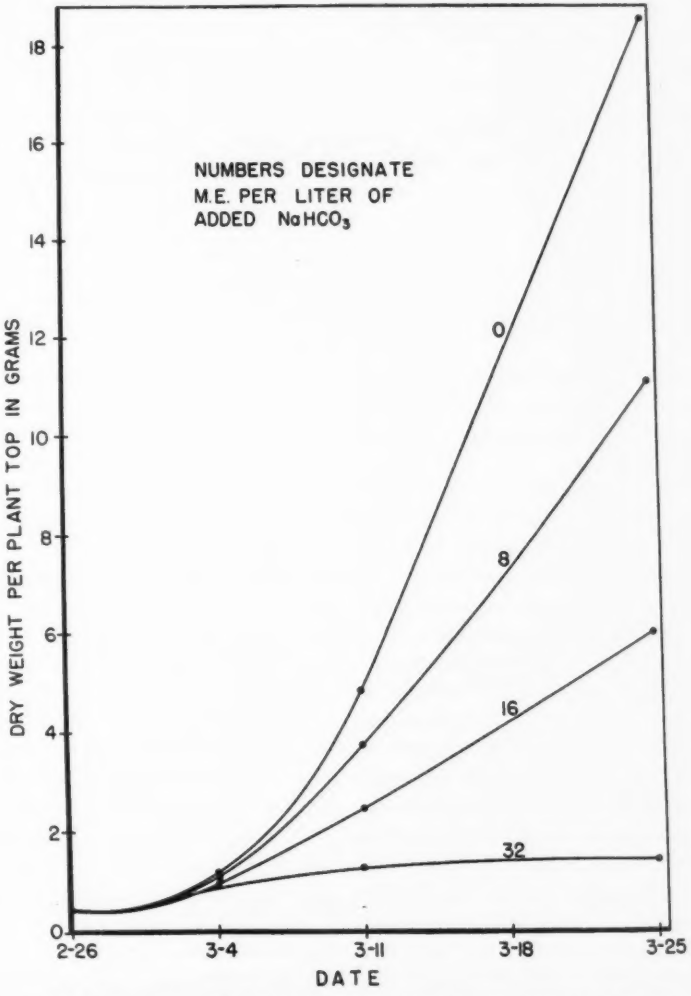

FIG. 1.—Growth response of Red Kidney bean plants to bicarbonate ion in culture solution

ly free of bicarbonate. Plants in the series with 32 m.e./l. added $NaHCO_3$ made virtually no further growth after the first week of treatment. Intermediate levels of added $NaHCO_3$ resulted in intermediate degrees of growth response. This inhibition in growth was due to the effect of the bicarbonate ion, since pH value of the substrate was eliminated as a variable. Earlier studies (**15**) have indicated that Na_2SO_4 and NaCl added to a control nutrient solution at levels isosmotic to the $NaHCO_3$ treatments should have a comparatively small effect in depressing growth of Red Kidney beans. Hence, growth responses under consideration must be interpreted as largely due to the specific toxicity of the bicarbonate ion with osmotic effects of the added solute playing a minor role.

TABLE 1

CHLOROPHYLL CONTENT OF TRIFOLIOLATE LEAVES, MG/100 SQ CM

HARVEST DATE	TREATMENT, M.E./L. $NaHCO_3$			
	0	8	16	32
3/4.....	2.25	2.41	1.54	1.00
3/11.....	3.01	2.54	2.25	0.75
3/25.....	3.65	2.97	1.92	0.31

Plants on the culture solution with 32 m.e./l. added $NaHCO_3$ were showing appreciable chlorosis of the trifoliolate leaves 1 week after initiation of treatment and had developed severe chlorosis by the end of the experiment. A moderate degree of chlorosis was evident in the trifoliolate leaves of plants in the series with 16 m.e./l. added $NaHCO_3$. This condition did not change consistently during the course of the study. Chlorosis was not evident in plants in cultures with 8 m.e./l. added $NaHCO_3$. The observations on chlorosis are reflected by the determinations for chlorophyll per unit of leaf (table 1).

On the basis of these data there was a relatively lower level of chlorophyll in the trifoliolate leaves even when only 8 m.e./l. $NaHCO_3$ were added to the nutrient solution. These observations on chlorophyll content are directly related to the effect of the bicarbonate and completely unrelated to the addition of sodium per se. Observations at this laboratory indicate that additions of NaCl and Na_2SO_4 to the substrate actually tend to enhance the chlorophyll content of bean leaves, presumably as a result of physiological drought.

Addition of bicarbonate to the substrate had relatively little effect upon the chlorphyll content of the primary leaves. Over the course of the study there was a general tendency toward an inverse relation between the chlorophyll content and the level of added $NaHCO_3$, but the differences were comparatively small.

MINERAL CONTENT.—The chlorosis was related to a disturbance in iron availability within the leaves. This was checked by spraying a dilute $FeSO_4$ solution on the leaves of seriously chlorotic plants which had resulted from use of a nutrient solution high in bicarbonate on a few cultures of plants not included in the experimental setup. This spray effected the reappearance of chlorophyll in chlorotic leaf tissue. Iron solution was not sprayed on experimental plants, and the iron content of their leaves showed appreciable variations that were in some degree correlative with treatment (table 2). Observations on total iron were somewhat erratic, and it is possible that merely washing the leaves with water was not adequate to remove iron present in surface-adhering particles, as shown by JACOBSON (**29**). At the final harvest it may be that the washing procedure

was more effective, since both total- and soluble-iron content showed a close relationship to chlorophyll content as conditioned by treatment. The trends in these data are consistent with observations by JACOBSON (29) and MCGEORGE (32) supporting the concept of OSERKOWSKY (36) that active iron in the leaves is a primary variant associated with this type of chlorosis. Furthermore, the data suggest that the modified status of iron in bean leaves afflicted with bicarbonate-induced chlorosis is similar to that of other species affected with "lime-induced" chlorosis in the field (1).

TABLE 2

TOTAL AND "SOLUBLE" IRON CONTENT OF DRY TISSUE FROM TRIFOLIOLATE LEAVES, MG/GM

M.E./L. $NaHCO_3$ IN CULTURE SOLUTION	HARVEST DATE					
	3/4		3/11		3/25	
	Total iron	"Soluble" iron	Total iron	"Soluble" iron	Total iron	"Soluble" iron
0	0.127	0.096	0.167	0.085	0.192	0.115
8	.130	.069	.164	.095	.172	.101
16	.125	.069	.219	.088	.151	.095
32	0.123	0.043	0.214	0.065	0.144	0.048
Standard error					0.011	0.0039

Table 3 presents the data for the cationic content of the leaves. Marked changes in the mineral composition of the foliage took place with increasing age, and at any given time there were pronounced differences in composition induced by treatment.

In the primary leaves the calcium and magnesium contents tended to increase with age for all treatments. This trend usually accompanies senescence. The potassium content of these leaves tended to decrease with age excepting on the plants supplied with the highest level of $NaHCO_3$.

Significant quantities of sodium were found in the primary leaves from plants produced at the highest concentration of bicarbonate, and the accumulation of sodium showed a marked increase with age. The summation of cation equivalents in these leaves increased pronouncedly with age, but, at any given time of observation, higher levels of $NaHCO_3$ in the nutrient tended to be accompanied by a lower total content of cations.

Observations on the trifoliolate leaves provided somewhat similar data. Contents of all cations increased with elapsed time. At any given time of observation, higher concentrations of bicarbonate were associated with a markedly lower content of calcium, with a variable effect on magnesium but a tendency for an increase, and with an increase in the concentration of potassium in these leaves. Sodium was found in appreciable amounts only in leaves from plants subjected to the highest level of $NaHCO_3$, and its concentration in these leaves in-

creased markedly with age. The summation of the cation equivalents in the trifoliolate leaves varied extensively, depending on treatment and time of observation. The data in table 3 indicate that variations in growth and chlorosis among the different treatments were associated with marked variations in content of the major cations within the leaves.

root cells as a result of treatment, since there is a good possibility that some of the calcium so assayed was actually occluded on the surface of the roots (see HARLEY and LINDNER [20]). The relatively high level of magnesium that appeared to be in the roots of plants grown at the highest level of added $NaHCO_3$ may be similarly accounted for. The

TABLE 3

CATIONIC CONTENT OF BEAN LEAVES, M.E./100 GM. OF DRY MATTER

M.E./L. $NaHCO_3$ IN CULTURE SOLUTION	HARVEST DATE	PRIMARY LEAVES					TRIFOLIOLATE LEAVES				
		Ca	Mg	K	Na	Total cations	Ca	Mg	K	Na	Total cations
0	2/26	87	60	152	Tr*	299	.....	.....	.....	.....	.....
0	3/4	161	70	150	Tr	381	72	42	100	Tr	214
0	3/11	211	75	137	Tr	423	111	50	101	Tr	262
0	3/25	289	104	124	Tr	517	124	60	113	1	298
8	3/4	144	58	182	Tr	384	57	44	104	Tr	205
8	3/11	178	63	174	Tr	415	82	57	122	Tr	261
8	3/25	244	109	138	Tr	491	106	70	124	1	301
16	3/4	120	60	174	1	354	56	55	116	2	229
16	3/11	163	75	166	2	404	80	61	133	2	276
16	3/25	201	99	156	3	459	103	72	157	1	333
32	3/4	100	52	163	8	315	30	45	104	8	187
32	3/11	88	54	167	22	331	55	58	147	30†	290
32	3/25	124	88	255	44	511	68	106	192	70	436
Standard error‡	.....	.....	.....	.....	.....	.....	±2.4	±2.9	±3.9	.....	.....

* Tr = trace.

† Approximated.

‡ Trifoliolate leaves harvested on 3/25 from the three replicate cultures of a given treatment were assayed separately, and these data were used to provide a measure of experimental error.

It is of interest to consider the cationic content in the tissue of the roots and stems plus petioles as well as that of the leaves. Pertinent data are given in table 4 as obtained at the final harvest. Higher levels of bicarbonate in the nutrient solution appeared to be associated with higher concentrations of calcium in the root tissue—the reverse of the trends observed in the two types of leaves. It cannot be stated that the data actually show an accumulation of calcium in the

definitely lower content of potassium and the opposite trend for sodium with higher bicarbonate level were outstanding. Higher concentrations of NaCl in the nutrient solution have been found to induce this same effect on the potassium and sodium contents of bean roots (16). Healthy bean roots showed a capacity to retain sodium selectively away from aerial portions of the plant, but an accumulation of sodium in the roots was associated with a depletion of potassium,

presumably as a result of ionic displacement (16); however, bean roots that have been impaired by calcium unavailability lose their capacity to retain sodium selectively, with the result that it moves into the leaves (4).

The data in table 4 suggest that a

TABLE 4

CATIONIC CONTENT OF ROOTS AND STEMS PLUS PETIOLES AT FINAL HARVEST M.E./100 GM. DRY MATTER

Plant part	M.e./l. $NaHCO_3$ in culture solution	Ca	Mg	K	Na	Total cations
Roots..	0	23	59	123	13	218
	8	29	44	106	36	215
	16	58	55	67	142	322
	32	56	73	24	116	269
Stems plus petioles	0	35	21	127	2	185
	8	34	27	154	3	218
	16	42	40	160	18	260
	32	31	49	89	215	384

relatively high concentration of the bicarbonate ion may affect the capacity of bean roots to retain sodium. The roots of plants grown at 16 m.e./l. $NaHCO_3$ were found to contain the highest content of sodium, and the data for stems and leaves show that very little sodium moved upward into the aerial portions of the plant. In contrast, roots from cultures containing 32 m.e./l. bicarbonate were found to have an appreciably lower concentration of sodium than those of the aforementioned plants, yet relatively high accumulations of sodium were found in the stems plus petioles and leaves of plants on the 32-m.e./l. treatment. Hence, it seems reasonably probable that the bicarbonate ion at the highest concentration was affecting the capacity of these bean roots to retain sodium selectively.

In contrast with the observation on root and leaf tissue, there seemed to be no effect of treatment on the calcium content of stems plus petioles. Magnesium tended to accumulate in the latter tissue with higher levels of bicarbonate. A similar response has been reported for Dallis grass (17). There tended to be a higher potassium content of stems plus petioles with higher concentration of $NaHCO_3$ in the substrate, excepting that the highest level of added bicarbonate resulted in plants with a high concentration of sodium in the stem plus petioles together with a correspondingly low concentration of potassium, further suggesting the displacing action of sodium upon potassium accumulation in this species.

No tendency was observed for the summation of the cation equivalents to

TABLE 5

CONCENTRATION OF PHOSPHORUS IN TISSUES EXPRESSED AS M.E. H_2PO_4/100 GM. DRY TISSUE

Tissue	Time of observation	M.e./l. $NaHCO_3$ in culture solution			
		0	8	16	32
Primary leaves....	3/4	22.0	16.3	12.7	12.7
	3/11	20.3	9.0	7.0	6.7
	3/25	18.7	10.0	8.3	5.0
Trifoliolate leaves....	3/4	27.7	21.7	18.0	16.7
	3/11	22.7	18.0	15.7	12.3
	3/25	18.0	18.7	17.7	12.0
Stems plus petioles...	3/25	17.6	16.9	13.1	9.1
Roots.....	3/25	17.8	16.5	11.3	12.3

be constant within any organ studied for the treatments maintained.

Since phosphate/iron balance has been emphasized in relation to the development of chlorosis (2, 36), it is pertinent to consider the data for phosphate content (table 5). They show a marked

tendency for content of phosphate to be lower with higher levels of bicarbonate in the nutrient medium; that is, these data show the opposite trend of those obtained by OLSEN (35), which indicated higher intensities of chlorosis with greater content of phosphate in the tissues. OLSEN (36) concluded that the iron was precipitated by the phosphate along the vascular bundles of the chlorotic plants. Although phosphate did not accumulate with severity of treatment of these bean plants, precipitation of ferric phosphate within the tissues is still a possibility. BIDDULPH (2) obtained radioautographs of various tissues of bean plants grown in nutrient solutions inducing varying degrees of chlorosis and supplied with either radioactive iron or radioactive phosphorus. In treatments that brought on chlorosis, iron and phosphorus were shown to accumulate intensely in the roots and conductive tissues with relatively low levels in the leaf parenchyma. Even though the absolute amounts of these two elements were analytically indistinguishable between green and chlorotic plants, the radioautographs showed that distribution of these two elements within tissues differed markedly, depending on intensity of chlorosis. Even though the bicarbonate ion tended to depress entry of phosphate into the bean plants of the present study, BIDDULPH's observations suggest that precipitation of ferric phosphate may have taken place in the conductive tissues of plants afflicted with bicarbonate-induced chlorosis, but the present data provide no basis for establishing this point.

ORGANIC ACID CONTENT.—ILJIN (23, 24, 25, 26) studied the organic acid content of the leaves of many species of plants showing varying degrees of lime-induced chlorosis when growing on calcareous soil. He found that in the development of this type of chlorosis an unusual increase in citric acid takes place. As an extreme case, he reported the leaves of a chlorotic *Sambucus nigra* to contain seventeen times as much citric acid as leaves of a comparable healthy shrub. ILJIN supported the view that disturbances in metabolism conducive to the accumulation of citric acid were precursive to the development of lime-induced chlorosis in leaves. MCGEORGE (32) also found some tendency for this relationship to hold among chlorotic and healthy citrus leaves but to be much more pronounced within peach leaves. MCGEORGE (33) studied this relationship further and found that it held for dicotyledonous but not for monocotyledonous plants.

In the light of the foregoing it appeared desirable to ascertain whether bicarbonate-induced chlorosis was accompanied by shifts in organic acid accumulation. It is evident from the data (table 6) that bean leaves are high in organic acids, especially citric. No oxalic acid beyond a trace was found, and an appreciable quantity of unknown acids was always present. Total and citric acids tended to accumulate with age in both primary and trifoliolate leaves. Higher levels of bicarbonate in the nutrient solution were associated with lower accumulations of citric acid in the primary leaves but with greater accumulation in the trifoliolate leaves. It was shown in table 1 that added $NaHCO_3$ affected chlorophyll formation in the trifoliolate leaves, but only slight influence on the greenness of the primary leaves was observed though not reported. Even though the response observed herein is not pronounced, it is not in disagreement with ILJIN's observations on the relation between "lime-chlorosis" and citric acid accumulation. With the

exception that malic acid tended to accumulate in the trifoliolate leaves with age, observations on this component were not wholly consistent.

Discussion

The foregoing data have shown that higher concentrations of the bicarbonate ion in nutrient solution bring about marked changes in the internal status as well as in the growth and appearance of bean plants. The ultimate objective of a study such as this should be definition of the cause-effect sequence between treatment and growth response. The data are not adequate to establish the precise physiological channels through which observed responses took place. It has been possible to ascertain the degree of concomitance among the numerous criteria of internal status as well as between these criteria and treatment, on one hand, and growth response, on the other. Although a validly established correlation by no means indicates causation, it would indeed be hazardous to seek a cause-and-effect relationship between conditions that have no degree of concomitance. In other words, covariability may be a guide, though not infallible, to the channels of causation.

TABLE 6

ORGANIC ACID CONTENT OF BEAN LEAVES, M.E./100 GM. OF DRY TISSUE

M.E./L. $NaHCO_3$ IN CULTURE SOLUTION	HARVEST DATE	PRIMARY LEAVES				TRIFOLIOLATE LEAVES			
		Total	Citric	Malic	Unknown	Total	Citric	Malic	Unknown
0	2/26	179	67	36	76	.....	.....	.....	.....
0	3/4	290	195	37	58	132	54	24	54
0	3/11	393	238	35	120	188	72	34	82
0	3/25	454	282	31	131	243	105	39	99
8	3/4	290	160	53	77	137	54	25	58
8	3/11	390	229	51	110	195	70	44	81
8	3/25	435	258	34	143	246	119	46	83
16	3/4	309	144	53	102	151	67	25	58
16	3/11	405	201	56	148	244	92	56	96
16	3/25	457	248	36	163	277	132	49	97
32	3/4	274	125	72	77	138	52	27	59
32	3/11	314	122	78	114	232	105	32	95
32	3/25	389	248	34	107	304	158	56	90
Standard error*	.....	.....	.....	.....	.....	±3.8	±1.6	±1.8	±2.6

* Trifoliolate leaves harvested on 3/25 from the replicate cultures of a given treatment were assayed separately, and these data were used to provide a measure of experimental error.

In interpreting the data, the concentration of the bicarbonate ion in the nutrient solution was considered as the primary causal factor, since previous experiments (**15, 16**) involving the response of bean plants to high levels of sodium sulfate and sodium chloride did not induce the plant responses herein reported.

In order to evaluate quantitatively the interrelationships prevailing in the data obtained, they were reduced statistically by analysis of variance and regression to

obtain the summary values such as those presented in table 7. In developing this table, the total sums of squares for the variability among the twelve observations taken for the four levels of treatment at the three harvest dates after inception of treatment are designated as 100% of the variability observed for a given criterion. The figures in the first four columns of table 7 partition this total variability on a relative basis among four sources of variance. Column 1 shows the proportion of total sums of squares attributable to mean levels for the three dates of harvest, i.e., $(\Sigma y^2)_t$ shows the proportion of variance due to two degrees of freedom arising from *time* of harvest. Column 2, $(\Sigma y^2)_b$, shows the percentage of total sums of squares attributable to the single degree of freedom arising from pooled *regression* on level of bicarbonate in the nutrient solution. Column 3, $(\Sigma y^2)_d$, shows the relative amount of the total variance that is attributable to the two degrees of freedom arising from *differences* among individual regressions for the three dates of harvesting (see SNEDECOR [43:327]). Column 4, $(\Sigma y^2)_e$, shows the proportion of total sums of squares assigned to the six degrees of freedom for error. Significance of a given component of variance is

TABLE 7

RELATION OF VARIOUS CRITERIA OF PLANT PERFORMANCE TO LEVEL OF BICARBONATE SUPPLY

Dependent variable	$(\Sigma y^2)_t$ (%) (1)	$(\Sigma y^2)_b$ (%) (2)	$(\Sigma y^2)_d$ (%) (3)	$(\Sigma y^2)_e$ (%) (4)	b_1 (5)	b_2 (6)	b_3 (7)	b_a (8)	$(r_a)^2$ (%) (9)
Log_e dry wt.	59.1**	25.9**	14.5**	0.5	− .006	− .040	− .088	− .045	63.2**
Chlorophyll	3.6*	83.8**	9.9**	2.7	− .044	− .070	− .106	− .074	86.8**
"Sol" Fe	16.5*	65.7**	9.4	8.4	− .0015	− .0007	− .0021	− .0014	78.8**
Ca, prim. lvs.	36.4**	55.6**	7.3**	0.7	−1.91	−3.77	−5.13	−3.61	87.5**
Mg, prim. lvs.	83.5**	11.2*	>.1	5.3	− .48	− .55	− .59	− .54	67.6*
K, prim. lvs.	1.9	38.8**	44.3*	15.8	.16	.67	4.17	1.67	39.3**
Σ cations, prim. lvs.	79.0**	10.5*	5.0	5.5	−2.25	−2.94	− .10	−1.77	50.0*
Ca, trifol. lvs.	51.5**	44.9**	0.7	2.9	−1.25	−1.60	−1.68	−1.51	92.8**
Mg, trifol. lvs.	58.5**	17.4**	17.6	6.5	.12	.22	1.41	.58	42.0**
K, trifol. lvs.	39.2**	37.3**	19.8**	3.7	.14	1.37	2.58	1.36	61.4**
Σ cations, trifol. lvs.	71.7**	8.6**	16.2**	3.5	− .72	.96	4.53	1.59	30.3**
Ca/Mg, trifol. lvs.	7.8*	83.2**	1.2	7.8	− .031	− .036	− .042	− .036	90.2**
K/Ca, trifol. lvs.	11.3**	85.2**	.6	2.9	.064	.053	.061	.059	96.0**
K/Mg, trifol. lvs.	52.3**	4.9	18.7	24.1	− .003	.016	0	.004	10.0
K+Na/Ca+Mg	6.0*	87.7**	2.7	3.8	.019	.028	.029	.025	92.8**
Phosphate, prim. lvs.	20.8	53.8**	1.1	24.3	− .27	− .36	− .38	− .34	68.0*
Phosphate, trifol. lvs.	22.6*	63.3**	2.4	11.7	− .32	− .31	− .20	− .28	81.9**
Σ org. acids, prim. lvs.	82.6**	8.5	2.4	6.5	− .45	−2.46	−1.88	−1.60	48.9
Citric acid, prim. lvs.	64.6**	25.8**	6.7	2.8	−2.05	−3.70	− .96	−2.26	72.9**
Malic acid, prim. lvs.	41.9**	39.0**	16.4*	2.7	1.01	1.28	.082	.79	67.0**
Σ org. acids, trifol. lvs.	87.4**	6.8*	2.6	3.2	.21	1.54	2.03	1.26	54.7*
Citric acid, trifol. lvs.	80.7**	11.2**	6.3*	1.8	− .02	1.15	1.65	.93	57.7**
Malic acid, trifol. lvs.	67.2**	3.0	6.7	23.1	.09	− .09	.51	.17	9.1

* Significant at the 5% level.
** Significant at the 1% level.

designated by asterisks, according to the usual convention. Columns 5, 6, 7, and 8 present four categories of regression coefficients: b_1 lists regression on treatment at the March 4 harvest; b_2 indicates the relation as of data for March 11; and b_3 evaluates regression on treatment at the last harvest. The general trend with all harvests pooled is shown by regression b_a in column 8, and the proportion of variance listed in column 2, $(\Sigma y^2)_b$, is attributed to b_a. The units for all regression coefficients may be ascertained by reference to pertinent data previously presented. The relative variance associated with net divergence in trends of b_1, b_2, and b_3 from that of b_a is shown in column 3, $(\Sigma y^2)_d$. Column 9, $(r_a)^2$, shows the proportion of variance accounted for by b_a out of the total variance exclusive of that due to time of harvest. The latter column presents key figures for evaluating the closeness with which different criteria of plant performance varied with level of bicarbonate concentration.

The data given in figure 1 show exponential trends for growth with respect to treatment levels; hence, to treat growth data more effectively by the procedures used in constructing table 7, they were converted to $\log_e$ of dry weight. The data in the first line of this table show that this logarithmic conversion of growth response enabled all but 0.5% of the total variance to be accounted for by time of harvest and linear regressions. It is to be noted, however, that there is progressive change in the values for regression of $\log_e$ dry weight on treatment with duration of the experiment and that divergence among these individual time regressions was a highly significant contributor to the total variance.

The net effect of time of harvest and linear regression on treatment accounted for 97.3% of variance in the data for chlorophyll in the trifoliolate leaves. Variations in chlorophyll were directly related to bicarbonate level to a remarkably high degree (83.8% of variance), and the progressive changes in the size of the regression coefficients with time of harvest were also highly significant. This showed that the relation of chlorophyll content in the leaves to bicarbonate level became more pronounced with elapsed time.

The extractable iron content of the trifoliolate leaves also showed a close linear relationship with treatment, even though there was appreciably more unaccountable variation in this component than in the two previously mentioned entities.

Space does not permit a full discussion of all entries in table 7. The reader may examine and evaluate these data according to his interest. In evaluating the cationic contents of the primary and trifoliolate leaves, it should be emphasized that there was a markedly negative correlation in both types of leaves between calcium content and level of treatment. By contrast, potassium content was positively correlated with treatment to a rather high degree. Total cationic equivalents showed only little relation to treatment, and the correlation of magnesium content was relatively minor. These statistics emphasize that the effect of the bicarbonate ion on the cationic nutrients is expressed predominantly by a depression in calcium accumulation. HELLER *et al.* (21) also noted that the presence of $NaHCO_3$ in the culture medium was associated with a marked lowering in the calcium content of tomato plants.

Table 7 presents statistics for various cationic ratios as related to treatment. All those considered excepting the potassium/magnesium ratio show a very high

degree of covariance with treatment. The potassium/calcium ratio showed the highest correlation with treatment (96.0%) of any cationic component studied. This may have been anticipated, since the absolute values for calcium and potassium content of leaves were highly correlative with treatment. The high significance of the calcium/magnesium ratio is probably merely a reflection of the high degree of correlation between calcium content and treatment.

TABLE 8

RATIOS SHOWING PROPORTIONATE INCREASE IN DRY WEIGHT FOR SPECIFIED GROWTH PERIODS

M.e./l. $NaHCO_3$ in culture solution	First period (2/26–3/4)	Second period (3/4–3/11)	Third period (3/11–3/25)
0	3.13	3.98	4.68
8	2.94	3.26	4.20
16	2.76	2.53	2.64
32	2.55	1.34	1.11

The ratio of monovalent to divalent cations in the trifoliolate leaves is certainly very closely related to treatment, but it is probably only a reflection of the pronounced covariance between potassium-calcium and treatment.

Phosphate accumulation in the primary leaves showed very little relation to treatment, but in the trifoliolate leaves it is evident that the bicarbonate level depressed phosphate accumulation to a highly significant degree and that this effect varied little with time of observation.

In evaluating the relation of organic acid content of leaves with level of added $NaHCO_3$, it is noteworthy that no component in either type of leaf shows really high covariability with treatment. Citric acid content is consistent in that it is highly significantly correlated with treatment when tested for both primary and trifoliolate leaves, but the regressions are negative for the primary leaves and positive for the trifoliolates.

The array of statistics in table 7 tends to cast doubt that the bicarbonate treatments had a direct effect on organic acid accumulations. It is more likely that the significant correlations obtained are merely reflecting intercorrelation with other internal components that are directly related to treatment.

The concomitance between growth response and the status of certain internal components was quantitatively evaluated. To this end it seemed preferable to adopt an index of the dynamic character of growth as distinguished from a static appraisal such as that given by data for terminal dry weight. Accordingly, growth over a given interval was recorded as the ratio between the dry weight per plant at the end of the period to that at the start. The ratios derived from data presented in figure 1 are recorded in table 8. Although the time interval for the third period is twice that of previous two periods, it is of interest that there was no significant difference among period means of these ratios.

In correlating these data for rate of growth with those indicating internal status, it appeared unsound to use observations on a given component at either the beginning or the end of a specified growth period. Consequently, the mean of the initial and terminal figures for a given component were used in the correlations with rate of growth. Since there were no trifoliolate leaves on the plants harvested on Feburary 26, data involving contents of trifoliolate leaves during the first period of growth were based on the status of the primary leaves at the start of this period.

The statistics presented in table 9 were

derived similarly to those given in table 7. Since growth response is designated as the dependent variable in each instance, the column of figures showing the percentage of total variance in the dependent variable attributable to time of observation is omitted, because the value is uniformly 2.7% for all comparisons. Although growth is herein arbitrarily shown as the dependent variable, it adequately expressed the relation for each of the three growth periods, since the percentage of variance attributable to differences among regressions was nearly nil.

Rate of growth showed a remarkably close linear relationship with observations on extractable iron. This was probably due to intercorrelation, since the extractable iron may be logically interpreted as conditioning chlorophyll content (see next section).

TABLE 9

RELATION OF GROWTH TO STATUS OF CERTAIN COMPONENTS OF TRIFOLIOLATE LEAVES

Independent variable	$(\Sigma y^2)_b$ (%)	$(\Sigma y^2)_d$ (%)	$(\Sigma y^2)_e$ (%)	b_1	b_2	b_3	b_0	$(r_m)^2$ (%)
Chlorophyll	90.2**	.9	6.3	.70	1.31	1.32	1.29	92.7**
"Sol" Fe	80.9**	6.2	10.3	22.5	69.5	75.3	65.6	83.1**
Ca	84.4**	2.4	10.5	.028	.055	.065	.058	86.7**
Mg	67.5**	1.4	28.5	− .048	− .126	− .138	− .132	69.3**
K	87.1**	2.5	7.7	− .030	− .087	− .059	− .063	89.5**
Σ cations	13.6	61.8*	21.9	.013	.035	− .067	− .023	14.0
Ca/Mg	82.1**	2.8	12.4	1.13	2.23	2.71	2.39	84.3**
K/Ca	82.6**	8.6	6.1	− .52	− 1.36	− 1.97	− 1.50	84.9**
K/Mg	55.5	14.5	27.3	1.75	− 6.49	−11.4	− 7.95	57.0*
K+Na/Ca+Mg	88.6**	1.9	6.8	− 1.79	− 3.50	− 4.02	− 3.70	91.0**
Σ org. acids	74.8**	.9	21.5	− .032	− .046	− .059	− .054	77.0**
Citric acid	84.4**	1.7	11.2	− .007	− .096	− .084	− .084	86.7**
Malic acid	9.9	4.6	82.8	− .29	− .016	− .111	− .074	10.2
Ca/citric acid	87.3**	5.3	4.7	1.53	2.94	4.25	3.41	89.7**
Phosphate	76.3**	15.4*	5.6	.096	.233	.454	.280	78.5**

* Significant at the 5% level.
** Significant at the 1% level.

It is indeed striking that growth rate is such a close concomitant of chlorophyll content of the trifoliolate leaves. This relationship could be logically set forth as implying causation, since all energy involved in growth processes enters the plant through the catalytic activity of chlorophyll. The pertinence of the relationship is further emphasized by the fact that the average regression should be implied that growth is an actual physiological resultant of the independent variables so selected.

The statistics show that there was a high degree of concomitance between both calcium and potassium contents of trifoliolate leaves and growth rate, the relations being positive with calcium and negative with potassium. Growth rate was negatively related to magnesium content of these leaves but was unrelated to the summation of cation equivalents. In view of the preceding, it is to be expected that rate of growth would also

be closely related to certain cationic ratios. Data in table 9 show this to be the case. It is especially noteworthy that growth rate was found to be such a close concomitant of the ratio of monovalent to divalent cations.

In evaluating these close relationships between growth rate and the status of cations in the trifoliolate leaves, it must be recognized that the physicochemical properties of the colloidal constituents of protoplasm will be conditioned by the nature of the cationic swarms about the micelles. It has been demonstrated that the activity of certain enzyme systems is dependent on the nature of the cations present as well as the proportional distribution (**5, 34**); yet it cannot be unqualifiedly stated that growth rate was directly dependent upon the cationic status in the trifoliolate leaves, since it is possible that cationic status is merely intercorrelated with other factors that effected loss of chlorophyll and depression of growth. In other words, the cationic status in the tops may be merely a resultant of disturbed metabolism in the absorbing roots and not necessarily a primary causative factor in the observed growth responses. The data do not permit an unqualified conclusion to be drawn.

Similarly, even though a high degree of correlation was found between growth rate and phosphate content of the trifoliolate leaves (table 9), it cannot be stated that causation was involved, since the status of phosphate may be merely a concomitant of primary factors conditioning growth.

Total organic acids, and especially citric acid, showed a high degree of inverse concomitance of growth rate. ILJIN (**25**) considered citric acid accumulation as a predictor of the onset of chlorosis, but more likely it results from disturbed metabolism induced by any of several causes. As pointed out by BONNER (**3**), an accumulation of citric acid must indicate a block in the aconitase system. It is not known if the activity of this enzyme is conditioned by the status of cations in the substrate.

The growth rate was found to have a very high positive correlation with the calcium-citrate ratio in the trifoliolate leaves (table 9). The question immediately arises as to whether the lower calcium content that was associated with decreased growth also depressed the activity of aconitase and permitted citrate to accumulate. The answer is not available.

Although it was shown that rate of growth showed a high degree of covariance with several components of these bean plants, it was not possible to define the channels of causation. It was stated that considerable intercorrelation among the various components prevailed. Table 10 sets forth information on the latter point. For each comparison in this table, the component arbitrarily selected as the dependent variable is listed first, and the independent variable second. The data for percentage distribution of variances pertain to the dependent variables. The data in this table were calculated in the same manner as that described for table 7, excepting that the column of figures headed by b_i designates the regression coefficients obtained among means for times of harvest, and the r_i^2 values show the proportion of variance related to time of harvest that may be accounted for by linear regression on comparable means for respective independent variables. These data aid in ascertaining whether a given relationship has the same characteristics between times of harvest as between treatments at a given time.

It is evident that a reasonably close

TABLE 10

INTERCORRELATION AMONG VARIOUS COMPONENTS OF TRIFOLIOLATE LEAVES

Dependent variable	Independent variable	$(\Sigma y^2)_t$ (%)	$(\Sigma y^2)_b$ (%)	$(\Sigma y^2)_d$ (%)	$(\Sigma y^2)_e$ (%)	b_1	b_2	b_3	b_a	$(r_a)^2$ (%)	b_t	$(r_t)^2$ (%)
Chlorophyll	"Sol" Fe	3.6	73.2**	7.7	15.5	23.4	64.6	48.4	42.4	75.8**	20.5	95.5
Chlorophyll	Ca	3.6	80.4**	5.5	10.5	.031	.040	.060	.046	83.4**	.009	94.8
Chlorophyll	K	3.6	81.0**	0.3	15.1	− .034	− .046	− .041	− .041	83.9**	.010	87.2
Chlorophyll	K/Ca	3.6	79.8**	11.2*	5.4	− .65	− 1.32	− 1.68	− 1.18	82.7**	− .70	99.6
Chlorophyll	K+Na/Ca+Mg	3.6	87.3**	3.5	5.6	− 2.25	− 2.47	− 3.47	− 2.86	90.5**	− 2.24	91.3
Chlorophyll	Phosphate	3.6	57.3**	.2	38.9	.105	.205	.418	.198	59.4*	− .091	99.7
Chlorophyll	Citric acid	3.6	76.2**	3.5	16.8	− .012	− .053	− .064	− .053	79.0**	.005	78.8
Chlorophyll	Ca/citric acid	3.6	75.1**	13.4	7.9	1.78	2.10	4.59	2.66	77.9**	− .70	11.0
"Sol" Fe	Ca	16.5*	61.4**	14.3*	7.8	.0012	.0003	.0012	.0009	73.6**	.0004	99.2*
"Sol" Fe	K	16.5	45.2*	2.4	35.9	− .0008	− .0004	− .0008	− .0008	54.1**	.0005	95.1
"Sol" Fe	K/Ca	16.5**	68.6**	7.5	7.4	− .023	− .013	− .035	− .024	82.3**	− .033	96.3*
"Sol" Fe	K+Na/Ca+Mg	16.5**	65.3**	11.5	6.7	− .078	− .027	− .071	− .055	78.4**	− .109	96.5*
"Sol" Fe	Phosphate	16.5	50.2**	18.5	14.8	.0040	.0018	.0089	.0041	60.2**	− .0043	96.4
"Sol" Fe	Citric acid	16.5	42.9*	8.8	31.8	.0003	− .0006	− .0013	− .0010	51.6	− .0003	89.3
K	Ca	39.2**	39.7**	13.8*	7.3	− .05	− .83	− 1.44	− .90	65.4**	.86	98.0**
K/Ca	Ca	11.3**	82.2**	3.2	3 3	− .051	− .031	− .035	− .037	92.8**	− .013	92.5**
K+Na/Ca+Mg	Ca	5.7*	88.1**	.2	6.0	− .015	− .016	− .017	− .016	93.5**	− .004	97.6*
Phosphate	Ca	22.6*	59.6**	4.5	13.3	.24	.21	.12	.17	77.2**	− .098	92.4*
Citric acid	Ca	80.7**	10.8**	4.8	3.7	.08	− .59	− .94	− .58	55.8**	1.50	94.0**
Citric acid	K	80.7**	17.2**	.1	2.0	.93	.76	.62	.66	88.9**	1.77	98.8**
Citric acid	K/Ca	80.7**	8.9**	7.4*	3.0	− 1.7	20.2	26.0	13.7	46.1**	− 99.1	74.5**
Citric acid	K+Na/Ca+Mg	80.7**	12.1**	4.2	3.0	− 7.1	37.5	54.2	36.8	62.6**	−371	92.9**
Citric acid	Σ org. acids	80.7**	16.4**	.5	2.4	.78	.53	.77	.66	84.7**	.54	95.2**
Σ org. acids	Σ cations	87.4**	7.5**	2.0	3.1	.26	1.63	.45	.46	59.3**	.96	98.4**

* Significant at the 5% level.

** Significant at the 1% level.

relation exists between chlorophyll and extractable iron content. Since it is well established that iron in some degree of activity is essential for chlorophyll formation, one might even have anticipated a higher degree of concomitance. It was mentioned earlier that the authors were not completely satisfied with their technique for determining iron in leaves and that the results may be somewhat aberrant. It is reasonable to conclude that the relation here found between chlorophyll and active iron implies causation.

The content of chlorophyll in the leaves was found to be closely related to calcium content, inversely related to potassium content, and inversely related to the potassium/calcium ratio. LINDNER and HARLEY (30) and others (50, 51) have reported these same conditions prevailing in leaves showing lime-induced chlorosis. It is even more striking that such a high degree of concomitance should be found between chlorophyll and the ratio of monovalent to divalent cations. On the basis of the present study, it is not possible to state whether chlorophyll concentration was directly affected by cationic status or whether the condition of both resulted from parallel influences by a precursive cause. LINDNER and HARLEY (30) suggested on the basis of their data that relatively high potassium levels in leaves inhibit the enzyme responsible for chlorophyll formation, but BENNETT (1) regarded this as improbable.

The significant relationships that appeared to prevail between chlorophyll and both phosphate and citrate probably merely reflect the effects of other intercorrelations. Even though chlorophyll content was found to be definitely related to the ratio of calcium to citrate, a causative influence may not be inferred.

The content of extractable iron was found to be closely related to content of calcium and to the potassium/calcium and monovalent/divalent cation ratios, and, as is further shown in table 10, the status of potassium, as well as those of the aforementioned ratios, is closely related to content of calcium. Certainly, "activity" of iron is much more closely related to calcium than to potassium. The fact that the status of so many other components in the leaves was closely related to calcium lends additional weight to the inference that calcium accumulation was a primary variant within these plants.

The data do not suggest that phosphate accumulation affected the "activity" of iron in these plants, since the two components were found to be positively related. The "activity" of iron appears to have little relation to the accumulation of citric or other organic acids.

An extremely high degree of positive concomitance was found between citrate and potassium contents. This may in some degree suggest that relatively high potassium levels coupled with a depression in calcium tend to inhibit activity of aconitase. The concurrence of these observed effects suggests a basis for ILJIN's (25) observation that citrate accumulation is precursive to the development of chlorosis.

It is evident that citrate is the main variant in the total content of organic acids and that there is a very close relationship between accumulation of cations and total organic acids. The importance of organic acids in cation-anion balance has been previously emphasized (38, 47).

In recapitulating the observations made during this study, the authors conclude that the primary disturbance arises at the root surface as a result of the effect

of accumulations of bicarbonate upon the activities of the absorbing cells. OVERSTREET *et al.* (**37**) pointed out as a result of studies on absorption of tagged bicarbonate that appreciable accumulation of bicarbonate by roots has never been demonstrated. These observations indicate that the predominant anions in the bicarbonate cultures did not enter the roots. On the other hand, through a mass-action phenomenon a relatively high concentration of bicarbonate on the external surface of the root would be inhibitory to CO_2 diffusion outward. Respiratory activity is essential for the process of salt accumulation by roots (**22**), and it is conceivable that this and other processes would be disrupted if the end-product of respiration is intensified in the absorbing cells. Higher levels of bicarbonate maintained at the root surface are tantamount to higher levels of CO_2 pressure at elevated pH. JACOBS (**27**) found that alkaline solutions containing $NaHCO_3$ were as effective in producing intracellular acidity as slightly acidic solutions with increased CO_2 pressure. CHANG and LOOMIS (**8**) have shown the specific toxicity of high concentrations of CO_2 upon graminaceous plants growing in water cultures at pH 4.0. Absorption of nutrient elements was depressed with the most pronounced effect upon entry of potassium. However, they noted differences in response among different species.

Many components of the bean plants were affected by bicarbonate level in the substrate, but it was indicated statistically that the primary effect on the plants was to depress entry of calcium and to accentuate entry of potassium. The work of STEWARD and PRESTON (**45**) is pertinent in this connection. They studied the effect of pH value and components of the bicarbonate buffer system on the entry of bromide into potato disks and found that "the most effective component of the bicarbonate buffer system which suppresses the uptake of bromide is the bicarbonate ion itself." They further observed that increasing concentrations of $KHCO_3$ progressively depressed protein synthesis and oxidase activity and commented that "this is the only case yet encountered in these investigations in which an increased concentration of a potassium salt decreased the respiration and metabolism of potato discs. It is evident that this effect is due specifically to HCO_3 and H_2CO_3." The data also showed that potassium was absorbed even at the higher concentrations of bicarbonate that suppressed protein synthesis and bromide absorption.

The bicarbonate ion was found to depress the accumulation of iron in leaves to a degree similar to that observed for calcium. Enhanced CO_2 pressures on cells change protoplasmic consistency (**28**), and the data presented indicate that the permeability of bean roots to monovalent ions was enhanced but that to divalent ions was depressed. Analysis of the bean roots for iron might have shown an accumulation such as MCGEORGE (**31**) observed for seedlings grown on soils that induce chlorosis, but it is also likely that much of the iron so determined would have actually been occluded on the surface of the roots as ferric carbonate or ferric phosphate. The authors are inclined to the view that bicarbonate ion induced the chlorosis and growth depression of these bean plants through its action on entry and activity of iron and that other derangements in the chemical status of the plants are largely concomitant with the effect of iron absorption and activity.

It is of interest to consider the degree to which the foregoing results may apply

to lime-induced chlorosis in the field. Carbon dioxide evolved by roots or by microbial activity would react with $CaCO_3$ present in the soil to effect an increased concentration of HCO_3^- at the root surface, even though no change in average CO_2 content of the soil mass could be detected analytically. THORNE (46) found no relation between degree of chlorosis and content of $CaCO_3$ on a calcareous soil in Utah but he did observe that chlorosis was related to the degree of compaction prevailing in the soil. Since compaction would depress gaseous interchange and facilitate CO_2 accumulation, it is possible that the compacted soils that induced chlorosis also effected a higher concentration of bicarbonate at the root surfaces.

There is much evidence that lime-induced chlorosis is intensified by high moisture content of the soil (6, 7, 41). Theoretically, higher soil-moisture content should be inhibitive to gaseous interchange and conducive to the accumulation of the bicarbonate ion in calcareous soils. Even though REUTHER and CRAWFORD (41) found no relationship between CO_2 content of a sample of the soil atmosphere and the degree of chlorosis of grapefruit when the intensity of symptoms varied with soil-moisture content over an irrigation cycle, it is possible that present gross sampling procedures of the soil atmosphere do not necessarily represent the state of the $CaCO_3$–HCO_3–CO_2 equilibrium at the root-soil *interface*. Certainly, the observations of HARLEY and LINDNER (20) strongly support the evidence that the bicarbonate ion may be the primary malefactor under conditions effecting "lime-induced" chlorosis.

Summary

1. The response of bean plants to additions of 8, 16, and 32 m.e./l. $NaHCO_3$ to a base nutrient solution was compared with that of plants grown on base nutrient solution adjusted to pH 8.0 with NaOH.

2. Higher concentrations of sodium bicarbonate were associated with greater intensities of chlorosis and marked inhibition in growth.

3. The treatments primarily effected a lowered iron "activity" and calcium content of the leaves and an enhanced potassium content.

4. The accumulation of citric acid in leaves showing intensified chlorosis was found to be a direct concomitant of potassium content.

5. It is suggested that the primary effect of the bicarbonate ion was through its effect on protoplasmic consistency of the absorbing cells of the roots so that these bean plants showed an accentuated accumulation of monovalent cations and a depressed accumulation of divalent cations.

6. The relation of bicarbonate-induced chlorosis to lime-induced chlorosis in the field is discussed.

LITERATURE CITED

1. BENNETT, J. P. Iron in leaves. Soil Sci. 60:91–106. 1945.
2. BIDDULPH, O. The translocation of minerals in plants. (Symposia on the Mineral Nutrition of Plants.) Univ. of Wisconsin Press, Madison. 1951.
3. BONNER, J. Plant Biochemistry. Academic Press, Inc., New York. 1950.
4. BOWER, C. A., and WADLEIGH, C. H. Growth and cationic accumulation by four species of plants as influenced by various levels of exchangeable sodium. Proc. Soil Sci. Soc. Amer. 13:218–223. 1948.
5. BRAVERMAN, I., and MORGULIS, S. The inhibition of the adenosine triphosphatose activity of actomysin by magnesium ions. Jour. Gen. Physiol. 31:411–415. 1948.
6. BURGESS, P. S., and POHLMAN, G. G. Citrus

chlorosis as affected by irrigation and fertilizer treatments. Arizona Agr. Exp. Sta. Bull. 124. 1928.

7. Burtch, L. M.; Thorne, D. W.; and Wann, F. B. The effect of light, soil temperature, and soil moisture on high-lime chlorosis. Proc. Soil Sci. Soc. Amer. 13:394–398. 1948.
8. Chang, H. T., and Loomis, W. E. Effect of carbon dioxide on absorptions of water and nutrients by roots. Plant Physiol. 20:221–232. 1945.
9. Clarke, F. W. The composition of the river and lake waters of the United States. U.S. Geol. Survey Prof. Paper 135. 1924.
10. Comar, C. L., and Zscheile, F. P. Analysis of plant extracts for chlorophylls "a" and "b" by a photoelectric spectrophotometric method. Plant Physiol. 17:198–209. 1942.
11. Eaton, F. M. Irrigation agriculture along the Nile and the Euphrates. Sci. Mon. 69:34–43. 1949.
12. ———. Significance of carbonates in irrigation waters. Soil Sci. 69:123–135. 1950.
13. Fireman, M., and Reeve, R. C. Some characteristics of saline and alkali soils in Gem County, Idaho. Proc. Soil Sci. Soc. Amer. 13: 494–498. 1948.
14. Gauch, H. G., and Wadleigh, C. H. A new type of intermittently irrigated sand culture equipment. Plant Physiol. 18:543–547. 1943.
15. ———, and ———. The influence of high salt concentrations on the growth of bean plants. Bot. Gaz. 105:379–387. 1944.
16. ———, and ———. Effect of high concentrations of sodium, calcium, chloride, and sulfate on ionic absorption by bean plants. Soil Sci. 59:139–153. 1945.
17. ———, and ———. The salt tolerance and chemical composition of Rhodes and Dallis grasses grown in sand culture. Bot. Gaz. 112: 259–271. 1951.
18. Gieseking, J. E.; Snider, H. J.; and Getz, O. A. Destruction of organic matter in plant material by the use of nitric and perchloric acids. Ind. and Eng. Chem. (anal. ed.) 7:185. 1935.
19. Hageman, R. H., and Hartman, E. L. Injuries produced by saline and alkaline waters on greenhouse plants and the alleviation of alkaline injury by neutralization. Proc. Amer. Soc. Hort. Sci. 39:375–380. 1941.
20. Harley, C. P., and Lindner, R. C. Observed responses of apple and pear trees to some irrigation waters of north central Washington. Proc. Amer. Soc. Hort. Sci. 46:35–44. 1945.
21. Heller, V. G.; Hageman, R. H.; and Hartman, E. L. Sand culture studies of the use of saline and alkaline waters in greenhouses. Plant Physiol. 15:727–733. 1940.
22. Hoagland, D. R., and Broyer, T. C. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. 11:471–507. 1936.
23. Höfler, K. Über Kalkchlorose und Calciose im Jahre 1941 und W. S. Iljin's biochemische Untersuchungen. Phytopath. Zeitschr. 14: 192–203. 1944.
24. Iljin, W. S. Calcium content in different plants and its influence on production of organic acids. Bull. de l'Assoc. Russe pour les Recherches Scientifiques à Prague 7:43–76. 1938.
25. ———. Die Kalkchlorose des Pflanzen und ihre Biochemie. Jahrb. f. wiss. Botanik 90:464–498. 1942.
26. ———. Salze und organische Säuren bei Kalkpflanzen. Flora 37:265–299. 1944.
27. Jacobs, M. H. The production of intracellular acidity by neutral and alkaline solutions containing carbon dioxide. Amer. Jour. Physiol. 53:457–463. 1920.
28. ———. The effects of carbon dioxide on the consistency of protoplasm. Biol. Bull. 42:14–19. 1922.
29. Jacobson, L. Iron in the leaves and chloroplasts of some plants in relation to chlorophyll content. Plant Physiol. 20:233–245. 1945.
30. Lindner, R. C., and Harley, C. P. Nutrient interrelations in lime-induced chlorosis. Plant Physiol. 19:420–439. 1944.
31. McGeorge, W. T. Nutrient interrelations in lime-induced chlorosis as revealed by seedling tests and field experiments. Arizona Agr. Exp. Sta. Tech. Bull. 116. 1948.
32. ———. A study of lime-induced chlorosis in Arizona orchards. Arizona Agr. Exp. Sta. Tech. Bull. 117. 1949.
33. ———. Lime-induced chlorosis: Relation between active iron and citric and oxalic acids. Soil Sci. 68:381–390. 1949.
34. Muntz, J. A. The role of potassium and ammonium ions in alcoholic fermentation. Jour. Biol. Chem. 171:653–665. 1947.
35. Olsen, C. Iron absorption and chlorosis in green plants. Compt. Rend. Lab. Carlsberg, Ser. Chim. 21:15–63. 1935.
36. Oserkowsky, J. Quantitative relation between chlorophyll and iron in green and chlorotic pear leaves. Plant Physiol. 8:449–468. 1933.
37. Overstreet, R.; Broyer, T. C.; Isaacs, T. L.; and Delwiche, C. C. Additional studies regarding the cation absorption mechanism of plants in soil. Amer. Jour. Bot. 29:227–231. 1942.
38. Pierce, E. C., and Appleman, C. O. Role of ether-soluble organic acids in the cation-anion balance in plants. Plant Physiol. 18:224–238. 1943.
39. Pucher, G. W.; Wakeman, H. J.; and Vickery, H. B. Organic acids in plant tissues. Ind. and Eng. Chem. (anal. ed.) 13:244–246. 1941.
40. Reitemeier, R. F. Semimicroanalysis of saline soil solutions. Ind. and Eng. Chem. (anal. ed.) 15:393–402. 1943.

41. REUTHER, W., and CRAWFORD, C. L. Effect of certain soil and irrigation treatments on citrus chlorosis in a calcareous soil. II. Soil atmosphere studies. Soil Sci. **63**:227–240. 1947.
42. SAYWELL, L. G., and CUNNINGHAM, B. B. Determination of iron—colorimetric O-phenanthroline method. Ind. and Eng. Chem. (anal. ed.) **9**:67. 1937.
43. SNEDECOR, G. W. Statistical Methods, 4th ed. Collegiate Press, Inc., Ames, Iowa. 1946.
44. STABLER, H. Some stream waters of the western United States. U.S. Geol. Survey Water-Supply Paper 274. 1911.
45. STEWARD, F. C., and PRESTON, C. Effects of pH and the components of bicarbonate and phosphate buffered solutions on the metabolism of potato discs and their ability to absorb ions. Plant Physiol. **16**:481–520. 1941.
46. THORNE, D. W. Factors influencing the solubility of iron and phosphorus in chlorotic and non-chlorotic areas of Hyrum clay loam. Iowa State College Jour. Sci. **15**:433–445. 1941.
47. ULRICH, A. Metabolism of non-volatile organic acids in excised barley roots as related to cation-anion balance during salt accumulation. Amer. Jour. Bot. **28**:526–537. 1941.
48. WALL, R. F., and CROSS, F. B. Greenhouse studies of the toxicities of Oklahoma salt contaminated waters. Oklahoma Agr. Exp. Sta. Tech. Bull. T-20. 1943.
49. WALL, R. F., and HARTMAN, E. L. Sand culture studies of various concentrations of added salts upon the composition of tomato plants. Proc. Amer. Soc. Hort. Sci. **40**:461–466. 1942.
50. WALLACE, T. Investigations on chlorosis of fruit trees. II. The composition of leaves, bark, and wood of current season's shoots in cases of lime-induced chlorosis. Jour. Pomol. and Hort. Sci. **7**:172–183. 1928.
51. WALSH, T., and CLARKE, E. J. A chlorosis of tomatoes. Eire Dept. Agr. Jour. **39**:316–325. 1942.

THE FLORAL MORPHOLOGY OF CLETHRA ALNIFOLIA WITH SOME NOTES ON C. ACUMINATA AND C. ARBOREA[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 632

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Introduction

Clethra, comprising some thirty to forty species of primarily Asiatic and American distribution, is usually the only genus classified in the Clethraceae. On the basis of the valved capsule and its type of dehiscence, the attachment and structure of the seed, and the method of dehiscence of the anthers, the family has long been considered a member of the Ericales. It was raised to family rank by KLOTZSCH (**18**) on the basis of the choripetalous, actinomorphic, deciduous corolla, the dehiscence of the two-chambered anthers by means of apical clefts, a two- to three-parted stigma, a loculicidal capsule, and the lack of bud scales. Of all the families placed in the order, the Clethraceae has often been regarded as one of the more primitive. Morphological studies have been made of various groups of the Ericaceae (**1, 5–9, 14, 16, 22–25, 27**), some species of the Empetraceae (**15, 27**), Diapensiaceae (**14, 27**), and Epacridaceae (**1, 24, 27**). The present study was undertaken to elucidate as much as possible the relationships of the Clethraceae with these families on the basis of various characters of floral morphology. It is hoped that detailed examination of the morphological features of various members of the Ericales will aid in the further clarification of relationships both within the order and between it and related groups.

DRUDE (**10**) divided the genus *Clethra* into three sections:

SECTION I.—Stamens and pistil projecting for a considerable distance beyond the stellately

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expanded corolla. Deciduous shrubs. *C. alnifolia* L., *C. acuminata* Michx., and *C. barbinervis* Sieb. & Zucc.

SECTION II.—Stamens included but stigmas extending beyond the corolla; anthers with rather long clefts at the apex; each locule with a short beak at the base. Evergreen, low tree. *C. arborea* Ait.

SECTION III.—Stamens and pistil not projecting beyond the corolla; anther halves undiverged at the base. Evergreen trees. This is the largest section and contains the remaining species.

The three species which were studied—*C. alnifolia*, *C. acuminata*, and *C. arborea*—represent the first two sections; no material was available of the third.

Some details of anthers and ovules of an unnamed species of *Clethra* have been presented by ARTOPOEUS (**1**) and of ovules and seeds in *C. acuminata*, *C. alnifolia*, *C. arborea*, and *C. barbinervis* by PELTRISOT (**25**) and SAMUELSSON (**27**). HAGERUP (**14**) has contributed chromosome-number determinations of *C. arborea* and *C. alnifolia*. The anatomy of vegetative organs and the structure of pollen, ovules, and seeds of *C. alnifolia*, *C. arborea*, *C. tomentella*, and *C. tomentosa* were investigated by LECHNER (**19**) for the determination of the taxonomic position of *Clethra* and three other genera which are now placed in the Actinidiaceae (Dilleniales). The vascular anatomy of the flower of *C. alnifolia* was briefly considered by HANCY (**16**).

This paper presents observations on the organography, floral development, vascular anatomy and the anatomy of the floral organs, microsporogenesis, and megasporogenesis in *C. alnifolia*, together with some observations on *C. acuminata* and *C. arborea*.

Material and methods

Plants of *Clethra alnifolia* and *C. acuminata*, obtained from E. C. ROBBINS, Gardens of the Blue Ridge, Ashford, North Carolina, were grown in a greenhouse at the University of Chicago. Plants of *C. arborea*, obtained from W. B. Clarke and Company, P.O. Box 343, San Jose 2, California, were also grown in the greenhouse. Only *C. alnifolia*, however, has flowered to date. Floral material of *C. arborea* was collected by Dr. B. F. PALSER and by the author at Strybing Arboretum, San Francisco, California, with the kind permission of the supervisor, Mr. ERIC WALTHER. Material of *C. acuminata* was obtained by the same collectors from the Cumberland Falls area in Kentucky and, with the permission of Mr. ARTHUR STUPKA, Park Naturalist, from Great Smoky Mountains National Park, Tennessee.

Conant's modification of Navashin's solution was used for killing and fixing most of the collections. A formalin–acetic acid–alcohol solution was used for some collections in an unsuccessful attempt to obtain material that would section more easily. All collections were dehydrated in an ethyl alcohol–tertiary butyl alcohol series. Various treatments, including soaking in water, nitric acid, and safranin solutions, mixtures of other waxes with paraffin, etc., were used with the hope of achieving better sectioning. Imbedding in Fisher Tissuemat (60°–62° C. m.p.) without special preliminary treatment gave the best, but by no means completely satisfactory, results. In addition to the inherent hardness of the material, the many thick-walled hairs arising from the epidermis of the pedicel and calyx caused considerable trouble in sectioning, so that, whenever possible, the sepals and pedicel were removed before dehydration. Cross and longitudinal series, ranging in thickness from 6 μ to 15 μ, but mostly 10 μ, were cut. FOSTER's (**13**) tannic acid–ferric chloride and safranin stain was found to

be very satisfactory for most anatomical studies. Heidenhain's iron alum–hematoxylin counterstained with Orange G was used to confirm and add certain cytological details. All drawings except figures 2–8 were made with a camera lucida.

Observations

The descriptions of *Clethra alnifolia* that follow are based on both fresh material and sections, while those of *C. acuminata* and *C. arborea* are based primarily on sections.

CLETHRA ALNIFOLIA L.

FLORAL ORGANOGRAPHY.—From twenty to fifty flowers are borne in an erect, terminal raceme or panicle, each flower in the axial of a bract (fig. 1). The flower is pentacyclic, predominantly pentamerous (the gynoecium consists of only three undiverged carpels), actinomorphic, choripetalous, hypogynous, and perfect. The sepals are slightly reflexed (fig. 63), and the very basal portions are undiverged (fig. 18). A rather heavy pubescence covers the surface of both pedicel and sepals (fig. 2). Aestivation in the bud is quincuncial—that type of imbricate aestivation in which two members are wholly external, two wholly internal, and one is half-internal and half-external (fig. 27).

Five completely diverged petals alternate with the sepals, are wholly glabrous, and show an aestivation which is also quincuncial (fig. 27).

The ten stamens occur in two whorls of five each. The outer whorl is opposite the petals and arises at a slightly lower level than the inner whorl which is opposite the sepals. The completely glabrous filaments are only slightly enlarged at the base and are flattened laterally, having a rectangular shape in cross section (figs. 26, 27). In the bud they meet the extrorse anthers at a point approximately one-fourth of the distance from the apex of the anthers. Inversion of the anther occurs at anthesis, however, so that the morphological base becomes the apparent apex, and vice versa, and the stamens become introrse (figs. 4, 5) with the filaments meeting the anther near the base. The short clefts formed at the time of dehiscence are found at the apparent apex (i.e., the morphological base) (figs. 3, 5, 6). The anther has four thecae, two on each side of the connective, each pair becoming united into a single sac a short time before the pollen is shed. Some elongation of the filament occurs before anthesis, so that, in older buds, there is an outward and downward bend in the upper part of the filament as it approaches the anther (fig. 63). After complete elongation of the filament the total length of the anther is about one-fifth of that of the filament (fig. 3). The thecae closest to the connective extend farther downward toward the apparent base than the other thecae. The two halves of the anther are separated from each other at the apparent apex for about half their total length. At the apparent base they are undiverged and continue below the base of the thecae as a single, attenuated, sterile tip. The two halves spread apart at the point where they diverge (figs. 3, 6).

There are no appendages such as spurs or awns on the stamens which at anthesis are definitely exserted (fig. 3). The pollen is shed as single grains, not as tetrads as in many of the Ericales.

The ovary, which is heavily pubescent on its upper surface (figs. 7, 8), is essentially globular but has a depression at the top from which the style emerges (fig. 63). The style is straight except for a slight expansion just above the ovary and terminates in three short branches

with stigmatic surfaces at their tips. At anthesis the stigmas are located well below the level of the stamens, but the style soon elongates, elevating the stigmas to a position beyond the level of the stamens (figs. 3, 7, 8). The ovary is three-carpellate, with the three locules occurring opposite the two wholly external sepals and the one which is half-internal and half-external (fig. 28). The three pendant placentae arise from the upper portion of a central column (figs. 28, 63); thus the placentation is axile. Each placenta occupies about one-third of the locule area of a single carpel and bears ovules on the outer surface between it and the dorsal wall (fig. 28). There are about forty relatively small ovules per carpel. They are unitegmic, tenuinucellate, and closer to anatropous than any other condition (fig. 52).

A split develops in the tissues of the central column, above the level of the placentae, and extends radially in the plane of the placentae, thus becoming continuous with the locules. It continues into and through the style, forming a three-angled stylar canal (fig. 29). The angles of the canal correspond to the locules and are continuous with them, while the tissues between the angles correspond to the septa. The canal is almost completely closed in young buds but opens rather widely in older flowers. Loose, papillate cells cover the stigmatic surfaces.

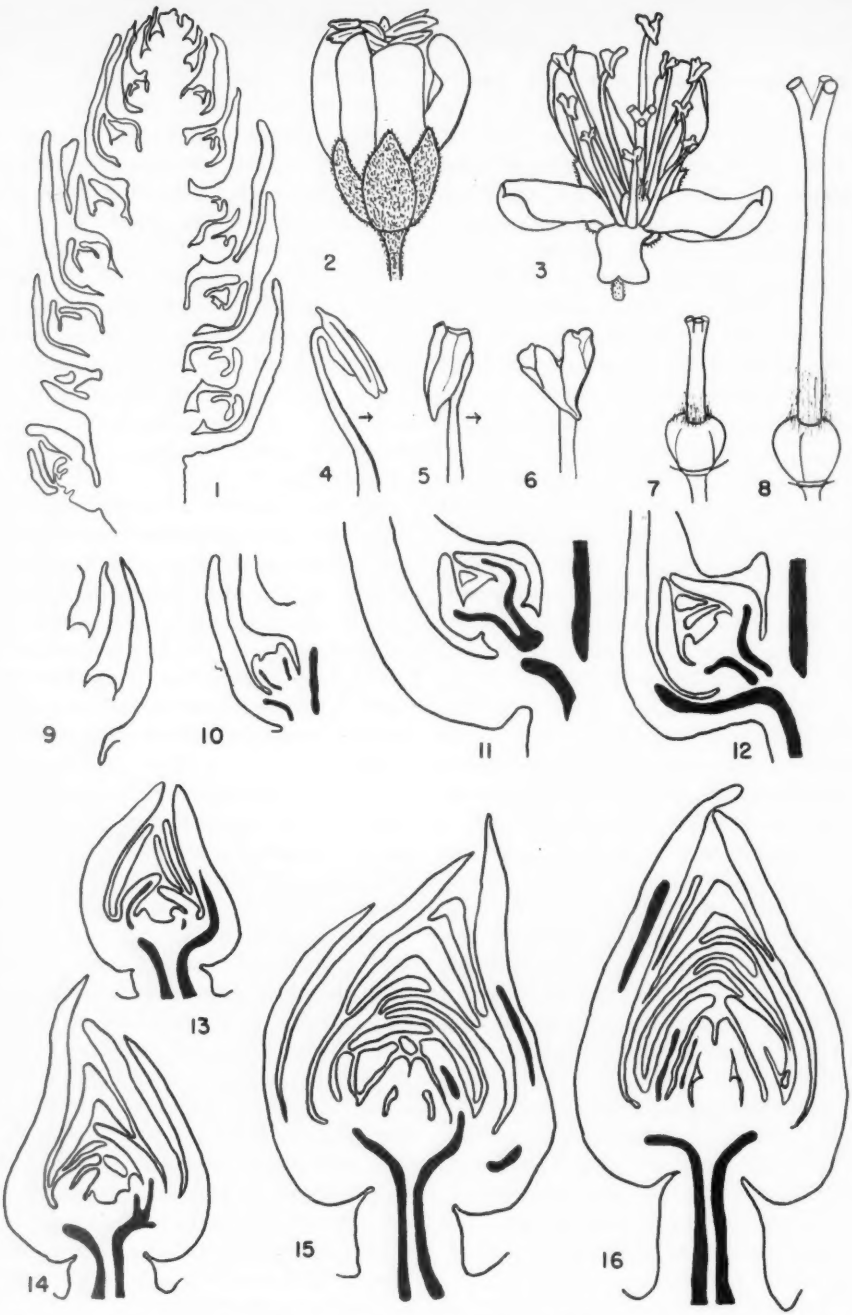
A relatively narrow zone on the outside of the very base of the ovary wall appears to be nectariferous in nature (fig. 25). Because there is no enlargement in the ovary wall in connection with this zone, a nectary of the simplest type is indicated.

FLORAL DEVELOPMENT.—Flowers are initiated in early spring and develop during spring and early summer, with anthesis occurring in midsummer. The order of appearance of individual flowers in the raceme or panicle is essentially acropetal (fig. 1) and spiral. Each individual flower appears as a blunt protuberance in the axil of a bract on the axis of the raceme or panicle (fig. 9). Growth is more rapid in circumference than in length, thus broadening the primordium. At the outer edge of the young apex the sepal primordia arise first, followed successively in an inward and somewhat upward direction by primordia of petals, stamens, and, finally, carpels (figs. 10–16).

By the time the archesporial cell in the ovule has differentiated, cells of the microsporogenous tissue are in initial phases of meiosis. When the archesporial cell has matured to form the megaspore mother cell, the reduction divisions of the microspore mother cells have been completed and the resulting tetrads separated into their constituent spores.

VASCULAR ANATOMY.—The vascular tissues of the peduncle and rachis of the raceme or panicle are in the form of a complete cylinder (fig. 17), that of the rachis broken by gaps left by traces to bracts and pedicels. Each bract below a flower receives a single trace, which leaves a single gap. Some branching of the bundle occurs within the bract. Each pedicel receives its vascular supply in the form of two traces; these arise on either side of the gap left by the bract trace and increase the size of the gap. The two bundles in the pedicel base soon form an essentially complete cylinder which traverses the rest of the pedicel to enter the receptacle where it broadens slightly before the traces to the floral organs arise (fig. 20).

In the receptacle it is difficult to determine just how many whorls of bundles



FIGS. 1–16.—Organography and floral development of *Clethra alnifolia*. Figs. 1–8, organography: fig. 1, longisection of young inflorescence, ×16½; fig. 2, flower bud at anthesis, anthers partially inverted, ×6½; fig. 3, flower, shortly after anthesis, ×3; fig. 4, stamen from a bud, prior to anther inversion; arrow points

arise because of the considerable compaction in the region of their origin. The bundles of the outer whorls, but particularly of that whorl supplying the sepals, curve downward in their course outward through the receptacle before turning up in a more normal position (fig. 63). The first whorl of bundles, five in number, forms the median supply of the sepals. Each median trace arises from the central cylinder and leaves a gap in the vascular ring (figs. 21, 22). The large strands of vascular tissue left between these gaps turn outward quite sharply immediately above the origin of the median sepal traces (fig. 23). The lateral traces of adjacent sepals arise as common bundles from the lower and outer surfaces of these strands. A common bundle divides radially in its downward and outward course, half passing to each of the adjacent sepals (fig. 18). Each sepal thus receives basically three traces, but, as the lateral traces often branch while still passing out to the sepals, the sepal base usually has six to seven traces (fig. 22). Occasionally, further branching occurs at higher levels in the sepals.

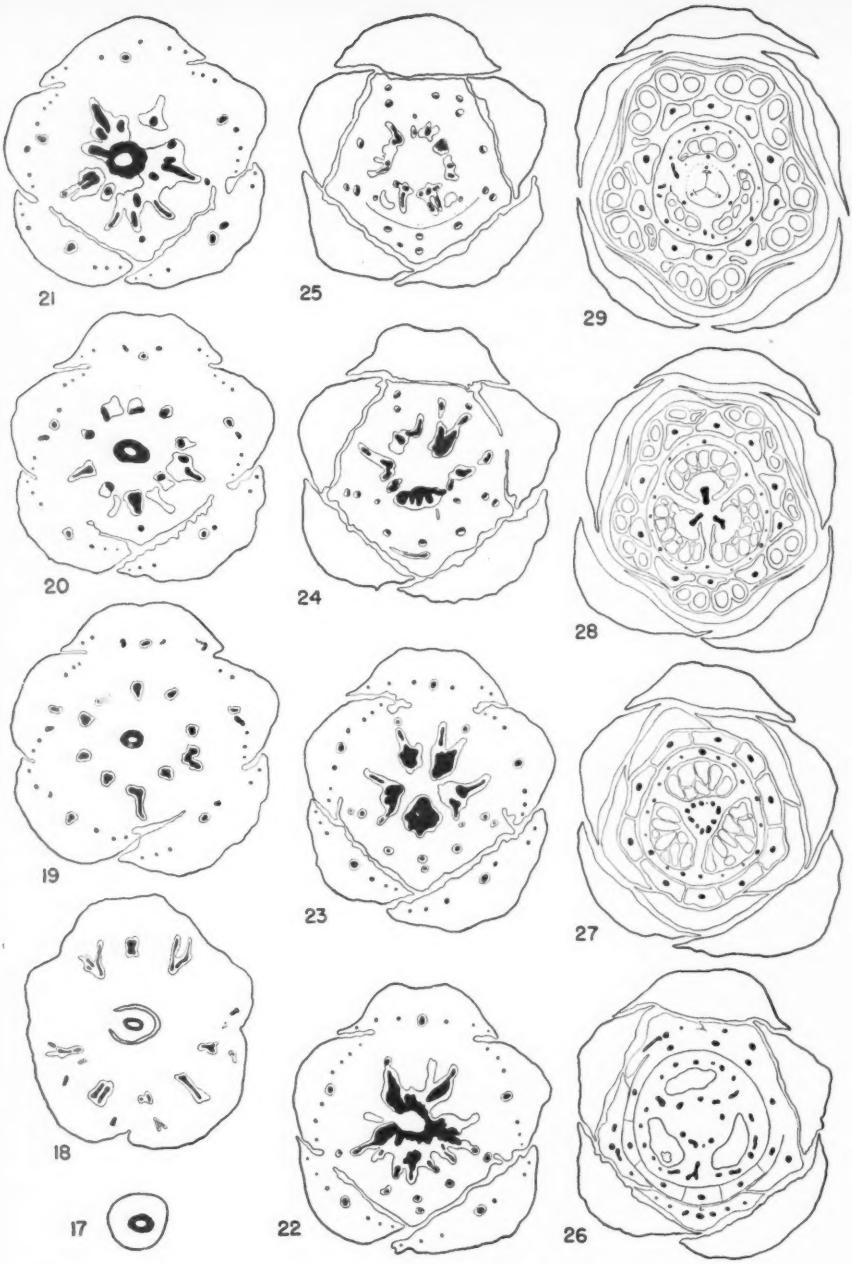
Each petal is supplied with a single trace which is derived close above the common lateral sepal traces, and thus alternating with the plane of the median sepal traces (figs. 19–22). A petal trace usually divides to form two to three strands in the base of the petal (fig. 24). Further divisions occur higher up, commonly resulting in nine bundles.

Traces to the petalad stamens have a common origin with the petal supply. A tangential division during the outward course of the common bundle gives rise to the petal strand on the outside and the stamen strand on the inside (figs. 21–24). Each stamen bundle passes undivided through the filament and into the connective of the extrorse anther, where it bends downward before terminating. A small strand essentially provascular in nature also extends upward in the connective above the bend (fig. 63).

Traces to the sepalad stamens arise from one side of the median sepal gap and curve outward to become aligned in the plane of the median sepal bundles. They appear to arise laterally from the vascular strands which gave rise first to the lateral sepal traces and then to petal and petalad stamen traces (figs. 21–24). There is no consistent pattern of orientation in the origin of a sepalad stamen trace with respect to the side of the gap from which it arises. The bundle in the sepalad stamen follows a course similar to that described for the petalad stamen.

The small dorsal carpel bundles originate in the plane of the two wholly external sepals and the one which is half-external and half-internal. Each arises from one side of a median sepal gap, usually from the side opposite the origin of the sepalad stamen trace, though occasionally it may diverge at a slightly higher level on the same side as the sepalad stamen trace (figs. 22–24). The dorsal carpel strands are not consistent with respect to the side of the gap from which they originate. They usually pass undivided through the carpel wall, turn under the depression at the top of the ovary, and enter the style where each is

outward, ×17; fig. 5, stamen from a flower, subsequent to anther inversion; arrow points outward, ×17; fig. 6, stamen from a flower, subsequent to anther inversion, showing apical dehiscence clefts, ×17; fig. 7, gynoecium from a flower, shortly after anthesis and prior to elongation of style, ×6½; fig. 8, gynoecium from a flower, about 1 day after anthesis and after elongation of style, ×6½. Figs. 9–16, floral development, ×41: fig. 9, young floral primordia; figs. 10–11, origin and development of sepals; fig. 12, origin of petals; fig. 13, origin of stamens; fig. 14, origin of carpels; fig. 15, locule formation; fig. 16, origin of placentae.



FIGS. 17–29.—Vascular anatomy of flower of *Clethra alnifolia*, ×16. Diagrams of serial cross sections of bud from pedicel to top of ovary. Figs. 19–25 are from sections 20 μ apart. In more critical areas of receptacle vascular tissue is outlined; solid areas within outlines indicate xylem.

located at the end of an angle of the stylar canal (figs. 29, 63). They continue through the style and its branches to a point just below the stigmas, where each one broadens considerably in a lateral direction and terminates.

Eight to twelve small septal carpel bundles, which leave gaps, arise above the level of the whorl of dorsal carpel strands and in the plane of the two wholly internal sepals and the petal between the half-external and half-internal sepal and the adjacent wholly external sepal. These septal bundles branch and rebranch, thus supplying the ovary wall between the septa and the dorsal carpel bundles (figs. 25, 26). The main septal bundles pass upward in the ovary wall in the plane of the septa to the top of the ovary, where they fade out without reaching the base of the depression or entering the style (fig. 29).

The remaining central vascular tissue of eight to twelve small bundles constitutes the ventral carpel supply (fig. 27). These small bundles turn quite sharply inward at first and then upward. They gradually meet to form an essentially continuous vascular ring which then redivides to form three bundles corresponding to the three locules. Each strand constitutes the total ventral supply of a single carpel and passes into the placenta, where it branches to form the vascular supply to the individual ovules (fig. 28).

The nectariferous tissue apparently receives no vascular supply. The main features of the vascular anatomy as described above agree with those presented by HANCY (**16**).

ANATOMY OF FLORAL ORGANS.—Just below the receptacle the stele occupies about one-fourth of the total diameter of the pedicel. The pith is small, and the cortex relatively large. Many cells in most tissues of the flower contain a darkly staining, homogeneous-appearing substance which is similar to that which other authors (**6, 17, 23**) have observed in other members of the order and have tentatively identified as tannin or a tannin-like material. This substance will be so considered in this paper also, although it has not been chemically identified. Many of the small, rather columnar epidermal cells contain tannin; others form long, thick-walled, unicellular, tanniniferous hairs which are usually found in groups of from two to five. The distal portions of these hairs project in all directions and constitute the pubescence—which other authors have called stellate—covering the pedicel. Frequently, two- or three-celled uniseriate hairs also occur. Each cell of a uniseriate hair usually contains tannin (fig. 60).

Cells of the cortical parenchyma are roughly isodiametric. They are relatively large and are only slightly separated by intercellular spaces. Many of them, located at random throughout the cortex, are filled with tannin.

The large size of cortical parenchyma cells makes it possible to distinguish cortex from stele. Identification of an endodermis and pericycle, however, is difficult. Elements of xylem and phloem are relatively small in cross section. Maturation of the xylem is endarch; arrangement of phloem with respect to xylem is collateral. Wall thickenings of xylem elements are both scalariform and spiral, though predominantly the latter. Rays, varying from one to two cells in width, are present. A large number of pith cells are filled with tannin, as are also scattered parenchyma cells in xylem, phloem, and rays.

Many epidermal cells of the abaxial surface of sepals are extended into thick-walled hairs similar to those on the

pedicel so that the outer surface of the sepals is thus also covered by a "stellate" pubescence. No hairs are found on the adaxial or inner surface, but here the columnar epidermal cells and a few of the oval hypodermal cells, particularly along the middle third of the surface, are thick walled. Nine to ten layers of chlorenchyma cells with some intercellular spaces occur at the point of maximum thickness in the center of the sepal. Many druses occur in the chlorenchyma cells close to the adaxial surface. The veins are located closer to the adaxial than to the abaxial surface. In them the xylem-phloem arrangement is typical: phloem toward the abaxial surface and xylem toward the adaxial. Cells containing a tannin-like substance are concentrated near the veins.

The abaxial epidermal cells toward the apex of the petals are papillate; those near the base are not. Most epidermal cells are tanniniferous; toward the apex of the petal other cells, usually concentrated around the veins, also show this characteristic. Both surfaces are covered with a fairly heavy layer of cutin which becomes fibrillated toward the apex of the petal. A greater size of the rather columnar, epidermal cells is noticeable particularly on the abaxial surface as compared with the adaxial, toward the base of the petal. The mesophyll is five or six cell layers thick and has little intercellular space. The number of layers decreases toward the apex. Veins show the usual orientation—xylem on the adaxial surface and phloem on the abaxial. The petals are completely glabrous. No crystals were observed in them.

Most of the epidermal cells of the filament are filled with tannin. A few internal cells also contain a similar material. The single unbranched vein shows the usual arrangement—xylem adaxially and phloem abaxially. In the connective of the extrorse anther the downward extension of the vein ending shows the reverse orientation. The upward extension shows the usual arrangement. The filaments are glabrous. A large number of epidermal cells of the anther as well as some cells of the connective and even an occasional cell of the endothecium are tanniniferous. Druses are found in the wall layers of the anther, in the cells of the connective, and in some cells of the endothecium (fig. 37). None was observed in the filament. The hypodermal layer forms a true endothecium by the thickening of its cell walls. The areas of endothecial development are shown in figure 36. Near the apparent apex a very narrow region between the two thecae of each anther half is characterized by very small cells and no endothecium. In this region short slits are formed when dehiscence occurs.

The ovary wall is eight or nine cells thick. The outer epidermis on the upper part and on the base of the style is characterized by a heavy pubescence. Hairs are of several types: very long, sharply pointed, thick-walled, unicellular or uniseriate hairs which are stellately arranged and contain tannin (fig. 62), and a few hairs which are similar but relatively thin-walled and occur individually. Two layers of cells lining the locules are elongated with their axes oriented on different diagonals so as to cross each other. The compact cells between the outer epidermis and the layers lining the locules are relatively undifferentiated, isodiametric, and parenchymatous. Tannin occurs in cells of the two inner layers, many cells of the outer epidermis, scattered cells of the wall, style, septa, placentae, and in the stigmatic papillae. The vascular traces are small. The orientation of the dorsal bundles, which con-

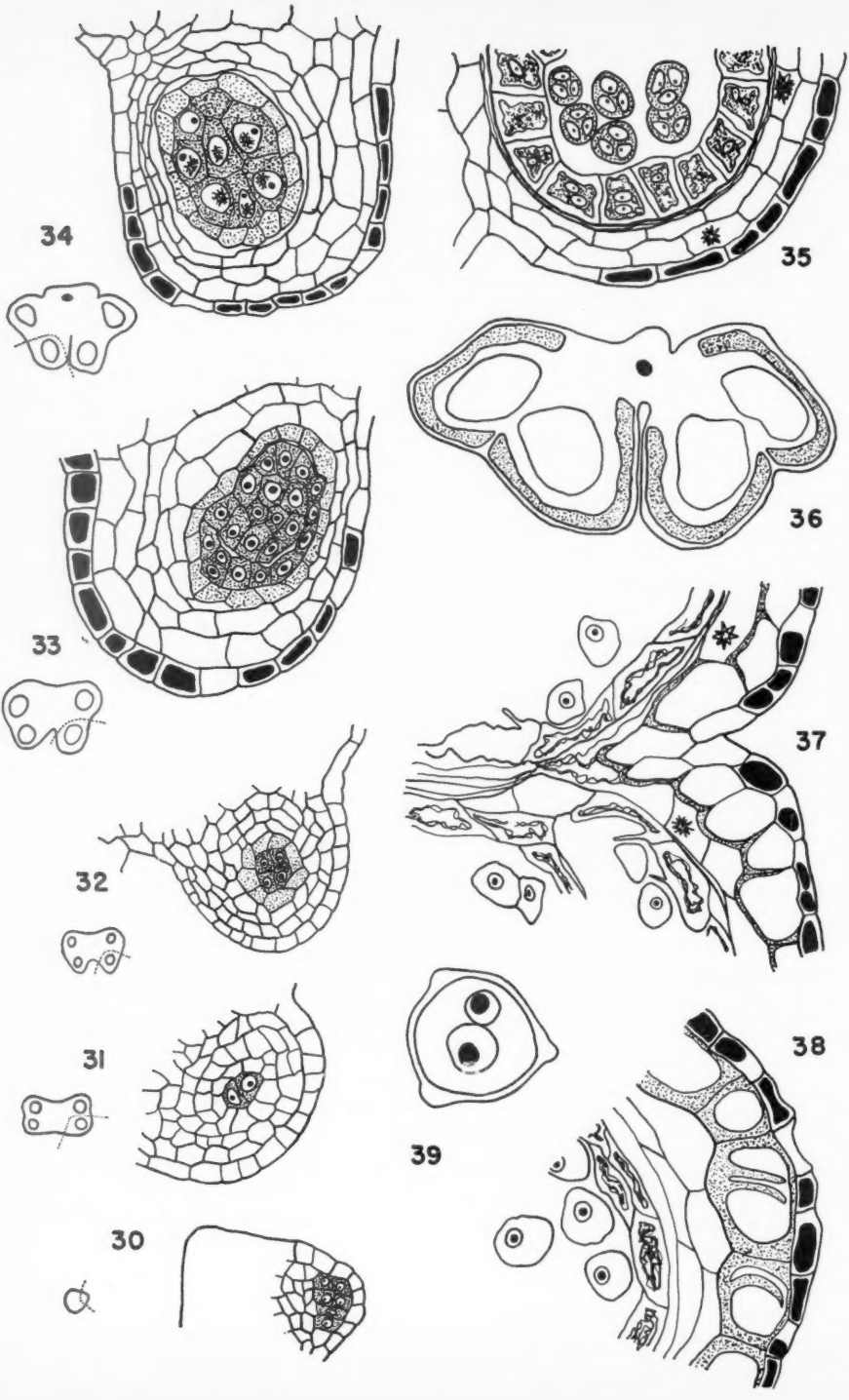
tinue as the stylar traces, is usual: xylem toward the center and phloem to the outside. The same is true of the septal bundles and their branches. The ventral bundles, however, show the reverse orientation: xylem toward the outside and phloem toward the inside. Cells of the massive placentae are parenchymatous, with little intercellular space. The canal of the cylindrical style is lined with small, compactly arranged cells. Most of the cells of the style are relatively undifferentiated, isodiametric, and parenchymatous. The three vascular bundles occur close to the ends of the angles of the canal (fig. 29). Each of the three stylar branches ends bluntly in a stigmatic surface covered with loosely arranged papillate cells. Druses occur in cells of the placentae and of the ovary wall next to the two layers which line the locules.

Cells of the nectariferous zone on the ovary base are small, compactly arranged, and somewhat granular. Few cells containing tannin-like materials appear in this zone.

Microsporogenesis.—The ten stamens arise immediately above the petals. The young stamen soon becomes distinctly four-lobed in cross section, each lobe representing a microsporangium. In each microsporangial region, as seen in cross section, several large hypodermal cells differentiate as archesporial cells, which soon divide periclinally to form a layer of primary parietal cells and a layer of primary sporogenous cells (fig. 30). The primary sporogenous cells undergo several successive divisions, forming a group of microspore mother cells from five to twenty in number in any one cross section (figs. 31–34). Meanwhile the primary parietal cells undergo periclinal divisions to form four layers. At about the time that the microspores are separating, the outer layer forms an endothecium. Thickenings develop on the inner and side walls of its cells as bands which extend perpendicularly to the longitudinal axis of the anther (figs. 37, 38). The thickenings occur in all cells of this layer except in a few between the two anther halves and in the area of actual dehiscence described earlier (fig. 36). The inner layer matures as a tapetum and then consists of large, isodiametric cells which are densely granular and usually binucleate (fig. 35). These cells remain intact until after the meiotic divisions have occurred, then disintegrate, and are absorbed by the developing spores.

The microspore mother cells undergo meiosis, and the microspores show a tetrahedral arrangement (fig. 35). The tetrads soon separate (figs. 37, 38). Within each spore the nucleus divides to form a generative and a tube nucleus. Separation into cells was not observed, but no attempt was made to stain especially for this point. This is the condition of the pollen at shedding. Each grain is rather flat, three angled, smooth walled, and has three points of emergence, one at each angle (fig. 39).

Megasporogenesis.—Small protuberances on the placental surface are the first indications of ovule development (fig. 40). The single integument arises very early as a small outgrowth toward the base of the nucellus, which at this stage constitutes the ovule. By the time the single hypodermal cell of the nucellus which constitutes the archesporium is recognizable by its large size, granular appearance, and greater affinity for stains, the integument is clearly discernible, and the ovule has begun to bend (fig. 41). The archesporial cell enlarges rapidly, mostly lengthwise, and becomes the megaspore mother cell directly. Con-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currently with these changes, elongation of the integument around the nucellus results in the formation of the micropyle. The ovule also increases considerably in size and completes the bend to become essentially anatropous (figs. 42, 43). The elongated spore mother cell, which is covered by a single layer of nucellus, undergoes meiosis, and a linear tetrad of megaspores is formed (figs. 44, 45). Three megaspores, usually those nearest the micropyle, disintegrate, and the remaining spore, usually the chalazal, increases rapidly in size (fig. 46). Occasionally the penultimate spore is the one which continues to develop (fig. 47). The nucellus begins to disintegrate almost simultaneously with the three nonfunctional megaspores (fig. 46). Even before this, however, the inner epidermis of the integument which closely surrounds most of the megagametophyte forms an "epithelial"-like layer characterized by narrowly columnar, compact, granular cells (figs. 45, 46). During gametophyte development the adjacent hypodermal layer often assumes some of these characteristics (fig. 51).

The functional megaspore becomes vacuolate as it enlarges, and its nucleus soon divides to form two nuclei which usually come to lie at either end of a rather large, central vacuole (fig. 48). Following another division the pairs of nuclei of the four-nucleate gametophyte are separated by the large central vacuole; smaller vacuoles occur in the cytoplasm surrounding the nuclei (fig. 49). Eight nuclei result from the next division. These three divisions occur in relatively close succession. Wall formation leads to the cutting off of seven distinct cells (figs. 50, 51). Thus, development is of the Normal-(*Polygonum*-)type (**20**). The mature megagametophyte occupies a little over half the entire length of the ovule and is of about the same width throughout its entire length (fig. 52). Fusion of the polar nuclei usually occurs before fertilization. The egg cell appears as an oval-shaped cap over the two rather slender synergids which are in close contact with each other and with the egg (fig. 51). The whole egg apparatus extends into the micropyle, and the adjoining epidermal cells of the integument appear to be displaced or somewhat crushed. The "epithelial" layer surrounds only the chalazal two-thirds of the gametophyte. The three antipodals are arranged in a linear row, the largest usually the farthest from the micropyle (fig. 51).

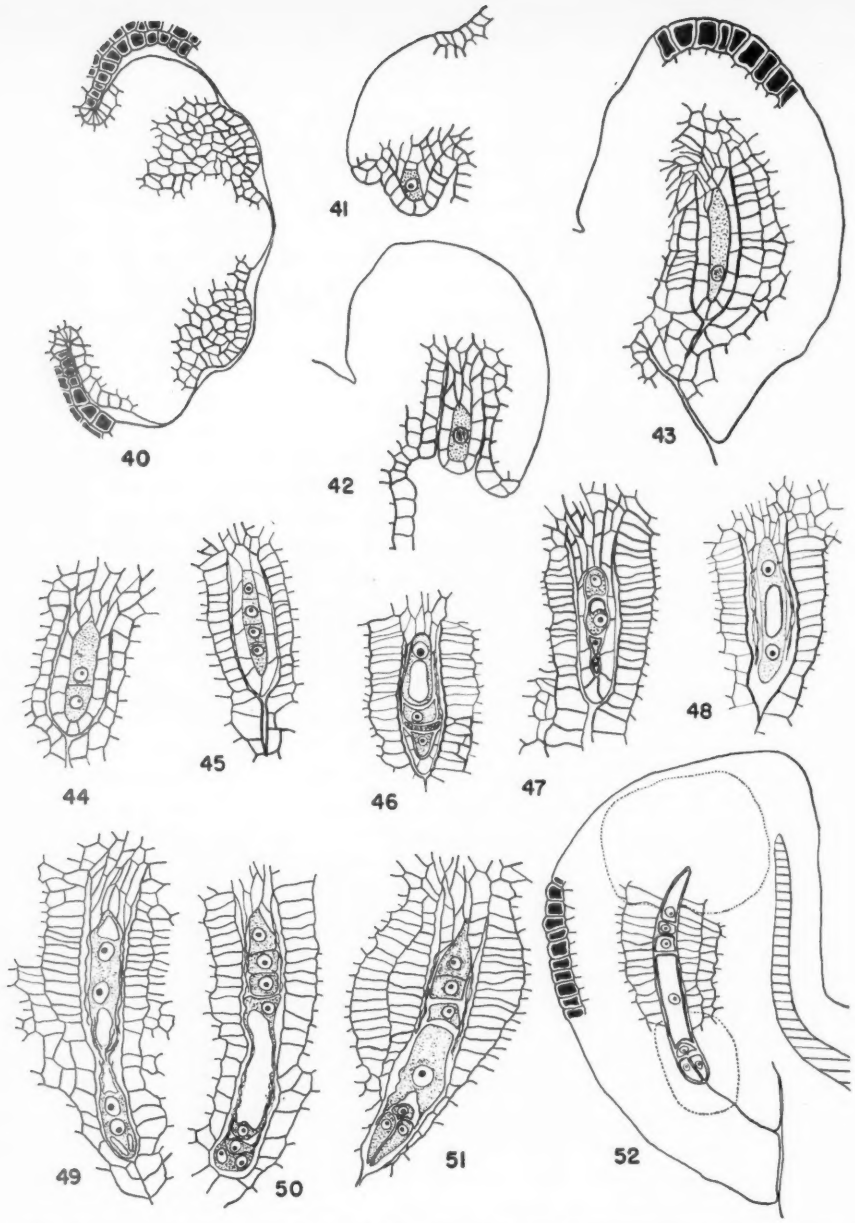
Areas of darkly staining, granular cells occur close to the megagametophyte in both the chalazal and the micropylar regions of the ovule (fig. 52). The chalazal area is the larger, and its limits are more easily discernible.

Difficulty in sectioning material of later stages of development prevented observations on fertilization, embryogeny, endosperm, and fruit development.

Clethra acuminata Michx.

Because of the limited amount of material of this species, only the organography and vascular anatomy of the flower and the anatomy of the floral

Figs. 30–39.—Anther structure and microsporogenesis in *Clethra alnifolia*, ×400 unless otherwise stated: fig. 30, cross section of portion of anther showing archesporial, primary parietal, and sporogenous cells; figs. 31–33, stages in development of sporogenous tissue and wall layers; fig. 34, microspore mother cells at beginning of meiosis; fig. 35, tetrads of microspores just prior to disintegration of tapetum; fig. 36, diagram of cross section of nearly mature anther showing areas of endothecium development, ×90; fig. 37, beginning of thickenings in endothecial layer; fig. 38, fully developed endothecium; fig. 39, pollen grain at time of shedding, ×900.



FIGS. 40–52.—Ovule development in *Clethra alnifolia*, ×331 unless otherwise stated: Fig. 40, origin of ovules from placenta in cross section, ×230; figs. 41, 42, formation and development of unicellular archesporium and of integument; fig. 43, megaspore mother cell; fig. 44, dyad; fig. 45, tetrad of megaspores and beginning of "epithelium" development; fig. 46, beginning of disintegration of three micropylar megaspores and of nucellus; fig. 47, penultimate chalazal megaspore functional; fig. 48, two-nucleate megagametophyte; fig. 49, four-nucleate megagametophyte; fig. 50, young eight-nucleate megagametophyte; fig. 51, mature eight-nucleate megagametophyte, polar nuclei fused; fig. 52, diagram of ovule containing mature megagametophyte and showing "epithelial"-like layer, darkly staining regions at micropylar and chalazal poles, and provascular strand supplying ovule, ×230.

organs were studied. There are many similarities between *C. acuminata* and *C. alnifolia* in these features. Certain recognizable differences are presented here.

The inflorescences are drooping, solitary racemes as compared with the upright racemes or panicles of *C. alnifolia*. In the individual flowers the filament base is oval in cross section rather than rectangular as in *C. alnifolia*. The filament of the latter species is glabrous, while in *C. acuminata* it is hairy. The type of hair on the filaments and on the surface of the upper part of the ovary differs from the stellately arranged, thick-walled, sharp-pointed hairs on the sepals, although both are extensions of epidermal cells. The hairs on the filament and ovary are relatively thin walled, unicellular or uniseriate, and their darkly staining contents are granular in appearance (fig. 61). Only a few hairs of this type were found on the ovary and style of *C. alnifolia*. In *C. acuminata* the style is glabrous.

The vascular supply varies from sepal to sepal within an individual flower (figs. 53–55): (*a*) for some a single trace leaves the central cylinder and separates radially into three main strands either in the base of the sepal or while still within the receptacle; or (*b*) a median trace arises independently and laterals diverge as in *C. alnifolia;* or (*c*) a median trace arises independently, one lateral diverges from a common bundle as in (*b*), and the other lateral is derived from the median trace as in (*a*). Branching of all traces occurs as higher levels are reached.

The adaxial epidermis of the sepal and three to four sublayers are composed of thick-walled cells. Toward the lateral edges of the sepal the number of layers of thick-walled cells is gradually reduced to just one, the epidermis.

One example was found of an increase in the number of members per floral whorl: a pistil with four carpels.

Clethra arborea Sieb. & Zucc.

The available material of this species was also limited, so that only the organography and vascular anatomy of the flower and the anatomy of the floral organs were studied. There are many similarities between *C. arborea* and the other two species in these features. Certain recognizable differences are presented here.

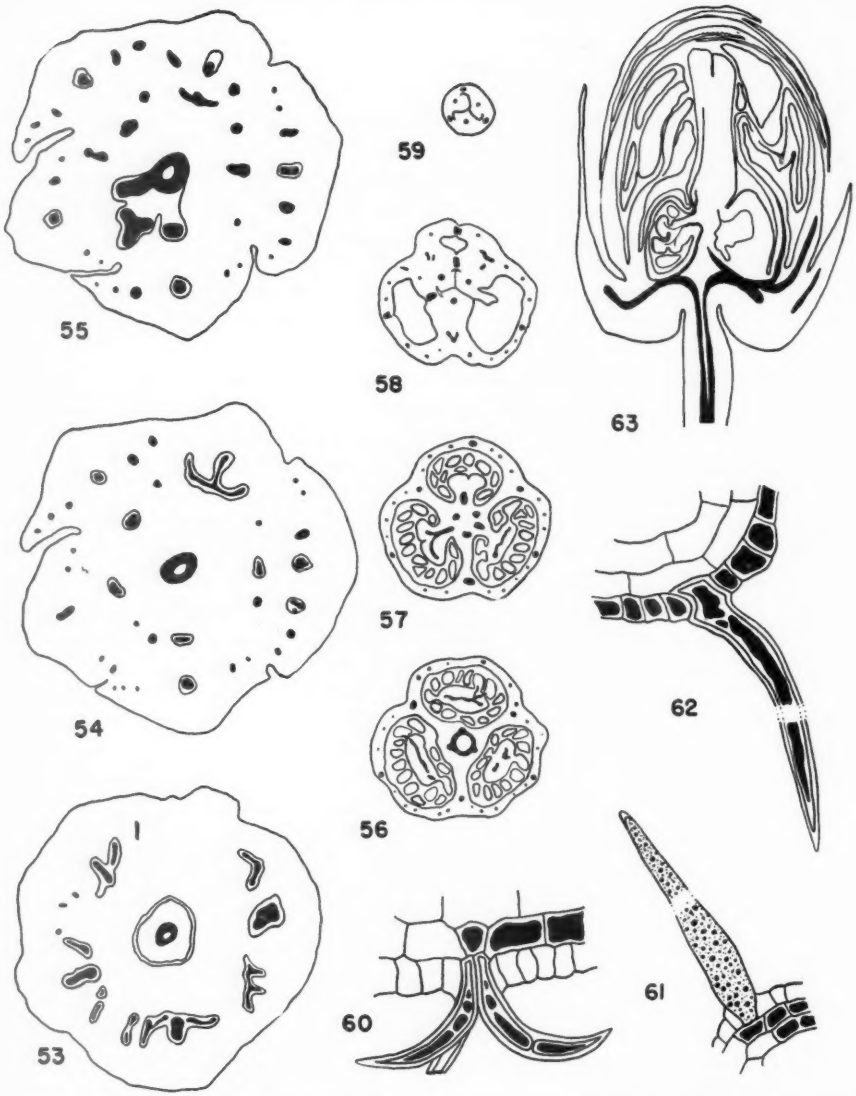
The inflorescences are upright racemes or panicles, as in *C. alnifolia*. The petals are much thicker than those of the two other species; at the petal base the mesophyll is twelve to fourteen layers thick.

The stamens are included at anthesis, and the filament base is triangular in cross section. The epidermal cells of the anthers are papillate and covered with a fibrillate cutin.

In contrast with *C. alnifolia* and *C. acuminata*, the ovules are borne on all surfaces of the pendant placentae rather than just on the outer surface next to the dorsal wall (fig. 56). The stylar canal is irregularly three-angled.

The vascular supply to the sepals is similar to that of *C. alnifolia*, except that often the median sepal bundle of the two wholly internal sepals and occasionally that of the half-internal and half-external sepal divides into two or three bundles before leaving the receptacle.

In the region of the ventral carpel bundles three small bundles in the planes of the septa of the ovary remain in the central column after the departure of the three placental traces from the essentially continuous vascular ring (figs. 56, 57). These three bundles continue through the style and its branches, ending at or just below the point where each dorsal carpel bundle broadens laterally and ter-



FIGS. 53–63.—Figs. 53–55, portion of vascular anatomy of flower of *Clethra acuminata*, ×24. Diagrams of serial cross sections of bud in region of origin of vascular supply to sepals. Where vascular tissue is outlined, solid areas within outlines indicate xylem. Figs. 56–59, *C. arborea*, diagrams of serial cross sections of upper portion of ovary and style, ×11. Fig. 60, *C. alnifolia*, stellately arranged epidermal hairs on surface of pedicel, ×240. Fig. 61, *C. acuminata*, epidermal hair on surface of ovary, ×240. Fig. 62, *C. alnifolia*, epidermal hair on surface of ovary, ×240. Fig. 63, *C. alnifolia*, diagram of longisection of bud, ×16½.

minates just below the stigmatic surface (figs. 58, 59). A cross section at any level of the style thus shows six bundles: three at the end of each angle of the stylar canal, the continuations of the dorsal carpel bundles, and the three strands described above which alternate with them. In both *C. alnifolia and C. acuminata* only the three continuations of the dorsal carpel bundles traverse the style.

Discussion

Clethra has rather consistently been placed in the Ericales, although occasionally as an anomalous genus. Most modern authors (**2, 3, 10, 12, 29**) have given it family rank, the status to which it was raised by KLOTZSCH (**18**) in 1851. ROBINSON and FERNALD (**26**), however, placed it in a tribe Clethreae of the subfamily Pyroloideae in the family Ericaceae. A possible relationship between *Clethra* and the Dilleniales was suggested by LECHNER (**19**) in her investigations of the morphology of *Saurauia*, *Actinidia*, *Clematoclethra*, and *Clethra*. The four genera were shown to have many features in common with both the Dilleniales and the Ericales. The characters of *Clethra*, however, were such as to lead her to separate it from the other three, which have since been placed in the Actinidiaceae (Dilleniales).

Many features of the Clethraceae ally it with the Ericales. Details for comparison between the species of *Clethra* described in the present paper and other groups of the Ericales are taken from ARTOPOEUS (**1**), COPELAND (**5–7**), DRUDE (**10**), FERNALD (**12**), HAGERUP (**15**), MATTHEWS and KNOX (**22**), PALSER (**23**), and SAMUELSSON (**27**). Characteristics of a general nature exhibited by most of the Ericales are: pentamery of most floral whorls, pentacycly, actinomorphy, hypogyny, and hermaphroditism. Those of a less general nature are the quincuncial aestivation of the calyx and corolla in the bud, the large amounts of tannin or tannin-like material, and the considerable number of druses.

Bilobed, unappendaged anthers, found also in the Rhododendroideae, are characteristic of *Clethra*. In the open flower, anthers of all investigated species in the order are introrse. Essentially all dehisce apically by means of short clefts, and the pollen grains are shed in the two-celled stage.

The essentially globular ovary, depressed at the summit with the style rising from the base of the depression, the stylar canal, the central placentae, the two layers of cells lining the locule which become fibrous in the course of fruit development, and the capsular nature of the fruit—all are characters of the gynoecium which the Clethraceae have in common with most other groups of the Ericales. The loculicidal dehiscence of the fruit is shared with some of the others—for example, the Andromedeae.

The many, small, tenuinucellate, unitegmic, anatropous ovules in which the nucellus disintegrates and the megagametophyte becomes surrounded by an "epithelial"-like layer differentiated from the inner epidermis of the integument are characteristics of all species which have been studied. Megagametophyte development according to the Normal-(*Polygonum*-)type (**20**) is an ordinal characteristic (**28**). Although the endosperm development and embryogeny of *Clethra* are not described in this paper, descriptions by others (**1, 21, 25, 27**) show that *Clethra* also corresponds in these respects to other members of the order. The endosperm is cellular throughout its development and forms both a chalazal and a micropylar haustorium. The embryo is rather small, straight, and

has two short cotyledons. It is imbedded in endosperm tissue and is surrounded by a seed coat which has been reduced to a single cell layer in thickness. The ovules of *Clethra* show areas of densely staining cells at both chalazal and micropylar ends of the gametophyte. Similar regions were reported by ARTOPOEUS (1) to occur in *Arbutus*, *Vaccinium*, and *Macleania* and were considered by him to serve as nutritive areas for the endosperm haustoria during seed development.

Those characteristics peculiar to *Clethra* are a fully developed endothecium in the anther and a three-branched style. *Bejaria*, *Rhodothamnus*, *Kalmia*, and *Phyllodoce* in the Rhododendroideae have a "more or less definite and extensive hypodermal layer of thick-walled cells in the anther" (6), but these cells are quite different in their characteristics from those forming the endothecium in *Clethra*. All investigated members of the order (except for *Clethra*) have unbranched styles, though the form of the stigmas may vary considerably. In *Dendrium* (*Leiophyllum*) the style ends abruptly in a flat stigmatic surface, while in *Azalea* (*Rhodora*) *canadensis*, *Kalmia latifolia*, and many others radiating lobes of the stigma equal the carpel number. Similarly, the Diapensiaceae have a three-lobed stigma, the lobes corresponding to the three-carpellate gynoecium.

There are certain other features which occur in the Clethraceae and in only a few other species and so are used in connection with those listed above to separate the family from other groups in the order. These include choripetaly, absence of a disk, "stellate" pubescence, extrorse position of the anthers during development, single pollen grains, and a tricarpellate gynoecium.

The majority of the Ericales are sympetalous, although there are choripetalous representatives other than *Clethra*. Examples are the Pyroleae, some of the Monotropoideae and Ericoideae, a few species of *Vaccinium*, and six genera in the Rhododendroideae.

Many of the Rhododendroideae and some of the Andromedeae have prominent disks, while others, such as *Pyrola*, have no disk. Certain members of essentially all groups are pubescent, the degree of pubescence and types of hairs varying considerably. None, however, is reported to have the *Clethra* type of pubescence, which consists of stellately arranged, unicellular and uniseriate hairs.

In most members of the order inversion of the anther occurs during the early development of the stamen, so that they become introrse long before anthesis (22). In *Cassiope*, however, such inversion occurs at a relatively later time than in most forms (23); while in *Clethra*, the Pyroleae (1, 7), and Arbuteae (1) inversion occurs at anthesis, so that the anther is extrorse during the entire development of the stamen and is introrse only in the open flower.

Pollen of the majority of the Ericales is shed in the form of tetrads, although in *Chimaphila* the tetrads are easily disrupted into single grains (7). Single pollen grains have been reported only in *Ramischia* (*Pyrola*) *secunda*, *Erica stricta*, the Monotropoideae, the Diapensiaceae, some of the Epacridaceae, and the Clethraceae (27).

The number of members within any whorl of an ericaceous flower is most commonly five, though variations (both up and down) occur. In *Clethra* the calyx, corolla, and androecium are pentamerous; the gynoecium, however, is trimerous. A few other members of the Ericales show a similar reduction in carpel number relative to the number of members

in the other floral whorls: the Diapensiaceae, and two members of the Phyllodoceae: *Loiseleuria* and *Dendrium*. The ovary in the Empetraceae is also tricarpellate, but here the number of members in other whorls is also reduced.

In the order as a whole the family Clethraceae has repeatedly been considered a primitive one (**1, 6, 7, 10**). This decision has been based primarily on such characters as pentacycly, actinomorphy, choripetaly, hypogyny, hermaphroditism, a true well-developed endothecium in the anther, the absence of a disk, and the fact that each carpel is represented by a separate branch of the style and stigma.

A branching style and the presence of a well-developed endothecium are usually considered to be primitive characters. A simple nectary in a group in which many forms have a well-developed one may also be considered to be primitive. This has been suggested by BROWN (**4**), who, in his study of the nectary, found that the lower part of the ovary in *Clethra lancifolia*, and in at least one representative of the Theales, is glandular and secretes nectar abundantly. He thought that the nectariferous condition shown by *Clethra* was intermediate between that of many of the Theales and that of a large number of the Ericales.

Choripetaly, usually considered to be primitive, has also been considered advanced, that is, derived by reduction. Thus COPELAND (**7**) has suggested that choripetaly in the Pyroleae and Monotropoideae can be interpreted as reduced, and so not primitive. It is possible that it might also be a derived condition in *Clethra*. In addition, COPELAND (**6**) suggested that the loculicidal dehiscence of the fruit of the Clethraceae is a derived character shared by the Andromedeae, Pyroleae, and some of the Monotropoideae.

With respect to the vascular anatomy of the flower, it is generally agreed that three traces with three associated gaps is the primitive condition of the vascular supply to a sepal (**7, 11, 23**). In the Ericales this type of sepal supply occurs in the Bejarieae, *Leucothoe racemosa*, *L. recurva*, and occasionally in *Chimaphila*, *Pieris*, *Neopieris*, and rarely in *Oxydendrum* (**6, 7, 23**), but it is not found in any of the three species of *Clethra* examined. PALSER (**23**) has suggested that

> two types of reduction have possibly occurred phylogenetically in the basic three-trace, three-gap sepal. In one the origins of the three traces to one sepal have come closer and closer together, until the three depart as a common bundle, leaving a single gap in the cylinder. This bundle separates to its three component strands while still in the receptacle. In the other type of reduction the laterals of adjacent sepals have come closer together in their point of origin, until they arise as a single bundle in the plane of the petal. Each pair of laterals thus leaves a single gap, and each median sepal trace a single gap, so that a total of ten gaps results in the vascular tissue of the receptacle. If, then, the internode between the lateral sepals and the petal traces were to become so compacted that these traces arose together, . . . the lateral sepal traces would appear to be derived from the petal traces [p. 476].

The pattern of the sepal supply in *C. alnifolia* approaches the latter condition, that is, the common lateral sepal bundle arises from the petal-petalad stamen bundle. In the sepals of *C. acuminata*, on the other hand, the vascular supply is such that this species occupies an intermediate position with respect to the two suggested lines of reduction, that is, the sepals show the vascular pattern characteristic of both lines and also a combination of the two types. *C. arborea* is more similar to *C. alnifolia* than to *C. acuminata* in this respect.

The petal trace in all species reported is a single bundle, as in *Clethra*. In some of the Rhododendroideae, Andromedeae, and Monotropoideae (**5, 6, 23**) the single trace divides to form three while still in the receptacle. In *Clethra* branching occurs only in the base of the petal or at an even higher level.

In all three species of *Clethra* examined the single stamen bundle bends downward in the connective before it terminates, as it does in all species described to date. A small strand, however, extends upward into the connective above the bend. The only other species in the order which has been reported to exhibit a similar condition is *Daboecia* (Rhododendroideae [**6**]).

Few reports have been found concerning the orientation in origin of the dorsal carpel strands in other genera having a two- or three-carpellate gynoecium. In *Dendrium* and *Loiseleuria* (both two- or three-carpellate) these strands arise from the remainder of the vascular tissue in the center of the flower above the departure of the stamen bundles (**6**), while in the three species of *Clethra* studied each strand arises from one side of a median sepal gap, that is, from the edge of the large mass of vascular tissue which supplies the petal and stamen traces.

Septal carpel bundles occur in all genera for which the vascular anatomy of the flower has been reported. In other genera besides *Clethra*, such as *Epigaea* and *Leucothoe*, they divide in their upward course, the small branches supplying the ovary wall between the septa and dorsal carpel bundles (**23**). In *Clethra*, however, none of these branches supplies the nectariferous tissue as they do in *Epigaea*.

The ventral carpel bundles provide the placental and ovule supply in all members of the order, but, in addition, in *C. arborea*—as in *Pyrola, Moneses*, and *Sarcodes* (**5, 7**)—a portion of each bundle continues into the style. Here these strands supplement the more usual stylar supply which is composed of the continuations of the dorsal carpel bundles.

The degree of nondivergence among the various strands to the floral organs is quite varied in the Ericales. In some of the Andromedeae (**23**) the petal and petalad stamen traces and the median sepal and sepalad stamen traces have a common origin, while in others all four of these traces arise independently. In similar fashion the dorsal and septal carpel traces may have an independent origin or arise in common with the corresponding stamen traces. The greatest degree of nondivergence among strands is seen in the epigynous Vaccinioideae. A completely independent origin of all traces seems indicative of primitiveness. In *Clethra* the degree of nondivergence is considerable. There is no occurrence in any of the three species studied of traces having a double origin; that is, an upper trace arising in two parts from either side of the gap formed by the departure of a lower trace—a feature accompanying the close origin of one trace above another, seen, for example, in some of the Andromedeae (**23**).

The entire picture presented by the vascular anatomy of the flower in the three species studied is one which indicates a considerable departure from the primitive condition.

A deviation from the most primitive is also suggested by HAGERUP's study of the chromosome numbers of various members of the Ericales (**14**) in which he presented a possible sequence of numbers that is very suggestive of the relationships among the forms which he studied. In this sequence $n=6$ is sug-

gested as the basic chromosome number from which a long series—$n=12$, 18, 24, 30, 36, and 48—has been derived by polyploidy. *C. arborea* ($n=8$) and *C. alnifolia* ($n=16$) form a short side series presumably derived from the basic number by the addition of two chromosomes. It is interesting that three species with low chromosome numbers—*Diapensia lapponica* ($n=6$), *Dendrium buxifolium* ($n=12$), and *Loiseleuria procumbens* ($n=12$)—have tricarpellate ovaries, as does *Clethra*, and that one, *Dendrium*, is also choripetalous.

Clethra, as exemplified by the three species studied, exhibits both primitive and advanced features which are of such a nature that it is difficult to determine which are the more phylogenetically important. Certainly the relatively advanced character of the vascular anatomy of the flower alone indicates that the family cannot be considered a primitive ancestral group so far as the other members within the order are concerned. It would seem, then, that *Clethra*, the Rhododendroideae, and probably other groups as well (see Cox, **8, 9**) have descended from a common ancestor which has disappeared together with any transitional forms, leaving a series of isolated survivors.

A study of the wood anatomy in the Clethraceae, such as made by Cox in the Rhododendroideae (**8**) and Arbutoideae (**9**) of the Ericaceae, might well help to clarify the relative degree of primitiveness or advancedness in *Clethra*.

DRUDE's separation (**10**) of the three species studied into two sections (I, *C. alnifolia*, *C. acuminata;* II, *C. arborea*) appears, on the basis of the usual taxonomic criteria, to be justifiable. Variations in the vascular anatomy and pubescence of the three species do not follow this separation entirely. It is only in *C. arborea* that branches of the three ventral carpel bundles traverse the style. The sepal supply, however, is different in all three, although those of *C. alnifolia* and *C. arborea* are fairly similar. The nature of the pubescence of *C. acuminata*, both in type of cell and in place of occurrence, separates it from the other two species. Neither of these differences, however, appears to be important enough to outweigh the criteria used by DRUDE. Before a really critical appraisal of this classification can be made, representative species of DRUDE's third section, which is the largest, and *C. barbinervis* (the third member of the first section) must be examined for comparison with the species described in this paper.

Summary

1. Descriptions of the organography, floral development, vascular anatomy of the flower, anatomy of the floral organs, microsporogenesis, and megasporogenesis in *Clethra alnifolia* and notes on the organography, vascular anatomy, and anatomy of floral organs in *C. acuminata* and *C. arborea* are presented.

2. The Clethraceae, in common with most of the Ericales, are pentacyclic (the outer whorl of stamens opposite the petals), pentamerous in most floral whorls, actinomorphic, hypogynous, and hermaphroditic. Characteristics peculiar to the family are a fully developed endothecium in the anther and a three-branched style. Other features found in the Clethraceae and in only a few other species in the Ericales are choripetaly, absence of a disk, "stellate" pubescence, extrorse position of the anthers during development, single pollen grains, and a tricarpellate ovary.

3. In the receptacle the vascular cylinder is considerably compacted in the region where the traces originate. The first

whorl, consisting of five bundles, forms the median sepal supply. The lateral traces of adjacent sepals arise as common bundles from the large strands of vascular tissue left between the median sepal gaps. Immediately above these common bundles the traces to the petals and petalad stamens arise together. Traces to the sepalad stamens arise from one side of the median sepal gap as do also the dorsal carpel bundles. The latter form the stylar vascular supply; the septal traces, independent in origin, fade out without reaching the style, and the ventral carpel bundles supply the placentae and ovules.

4. Inversion of the extrorsely developed anther occurs at anthesis. The pattern of microsporogenesis is as in most angiosperms. Pollen grains are single and probably two celled at the time of shedding.

5. In the ovule a single archesporial cell gives rise to the megaspore mother cell which, following meiosis, divides to form a linear tetrad. Usually the chalazal spore functions. The seven-celled, eight-nucleate, megagametophyte develops according to the Normal-(*Polygonum*-) type. Disintegration of the nucellus and development of an "epithelial"-like layer of the single integument begins prior to gametophyte development. Fusion of the polar nuclei occurs before fertilization.

6. *C. acuminata* and, to a lesser extent, *C. arborea* differ from *C. alnifolia* in the vascular supply of the sepals. Some variation in the type of pubescence occurs in *C. acuminata* as compared with the two other species. In *C. arborea* branches of the ventral carpel bundles as well as of the dorsal carpel bundles traverse the style.

7. A comparison of the characteristics of the Clethraceae, as exemplified by the three species studied, with those of other groups in the Ericales shows that the family is not so primitive with respect to the vascular anatomy of the flower as it is with respect to certain of its other morphological features.

8. DRUDE's separation of the three species studied into two sections appears to be justifiable on the basis of organography. Observed anatomical variations in the three species, however, neither support nor refute his classification.

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LITERATURE CITED

1. ARTOPOEUS, A. Über den Bau und die Öffnungsweise der Antheren und die Entwicklung der Samen der Erikaceen. Flora **92**:309–345. 1903.
2. BRITTON, N. L. Clethraceae. *In* North American Flora **29**:1–9. 1914.
3. BRITTON, N. L., and BROWN, A. An Illustrated Flora of the Northern United States, Canada and the British Possessions. Vol. 2. Charles Scribner's Sons, New York. 1913.
4. BROWN, W. H. The bearing of nectaries on the phylogeny of flowering plants. Proc. Amer. Phil. Soc. **79**:549–596. 1938.
5. COPELAND, H. F. Further studies on Monotropoideae. Madroño **6**:97–119. 1941.
6. ———. A study, anatomical and taxonomic, of the genera of Rhododendroideae. Amer. Midl. Nat. **30**:533–625. 1944.
7. ———. Observations on the structure and classification of the Pyroleae. Madroño **9**:65–102. 1947.

8. Cox, H. T. Studies in the comparative anatomy of the Ericales. I. Ericaceae—subfamily Rhododendroideae. Amer. Midl. Nat. **39**:220–245. 1948.
9. ———. Studies in the comparative anatomy of the Ericales. II. Ericaceae—subfamily Arbutoideae. Amer. Midl. Nat. **40**:493–516. 1948.
10. Drude, O. Clethraceae, Pirolaceae, and Ericaceae. *In* Engler, A., and Prantl. K, Die natürlichen Pflanzenfamilien. IV. Teil. 1 Abt. Leipzig. 1891.
11. Eames, A. J., and MacDaniels, L. H. An Introduction to Plant Anatomy. 2d ed. McGraw-Hill Book Co., New York. 1947.
12. Fernald, M. L. Gray's Manual of Botany. 8th ed. American Book Co., New York. 1950.
13. Foster, A. S. The use of tannic acid and iron chloride for staining cell walls in meristematic tissue. Stain Tech. **9**:91–92. 1934.
14. Hagerup, O. Morphological and cytological studies of the Bicornes. Dansk. Bot. Arkiv. **6**:1–26. 1928.
15. ———. Studies on the Empetraceae. K. Danske Vidensk. Selsk., Biol. Meddel. **20**:1–49. 1946.
16. Hancy, A. J. Vascular anatomy of certain ericaceous flowers. Cornell Univ. thesis. 1916.
17. Johansen, D. A. Plant Microtechnique. McGraw-Hill Book Co., New York. 1940.
18. Klotzsch, J. F. Studien über die natürliche Klasse Bicornes. Linnaea **24**:1–88. 1851.
19. Lechner, S. Anatomische Untersuchungen über die Gattung *Actinidia*, *Saurauia*, *Clethra* und *Clematoclethra* mit besonderer Berücksichtigung ihrer Stellung im System. Beih. bot. Zentralbl. **32**:431–467. 1914.
20. Maheshwari, P. An Introduction to the Embryology of Angiosperms. McGraw-Hill Book Co., New York. 1950.
21. Martin, A. C. The comparative internal morphology of seeds. Amer. Midl. Nat. **36**:513–660. 1946.
22. Matthews, J. R., and Knox, E. M. The comparative morphology of the stamen in the Ericaceae. Trans. and Proc. Bot. Soc. Edinburgh **29**:243–281. 1926.
23. Palser, B. F. Studies of floral morphology in the Ericales. I. Organography and vascular anatomy in the Andromedeae. Bot. Gaz. **112**: 447–485. 1951.
24. Payer, J. B. Traité d'organogénie comparée de la fleur. Paris. 1857.
25. Peltrisot, C. N. Développement et structure de la graine chez les Ericacées. Jour. de Bot. **18**:309–367; 386–402. 1904.
26. Robinson, B. L., and Fernald, M. L. Gray's New Manual of Botany. 7th ed. American Book Co., New York. 1908.
27. Samuelsson, G. Studien über die Entwicklungsgeschichte der Blüte einiger Bicornes-Typen. Svensk. Bot. Tidskr. **7**:97–188. 1913.
28. Schnarf, K. Embryologie der Angiospermen. Borntraeger, Berlin. 1929.
29. Small, J. K. Manual of the Southeastern Flora. New York. 1933.

REPRODUCTIVE ACTIVITY IN BROMUS INERMIS IN RELATION TO PHASES OF TILLER DEVELOPMENT[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **633**

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Introduction

Many investigations have shown the interrelationships between developmental physiology and morphology and ecological conditions in determining the change-over from vegetative to reproductive activity in higher plants (**6, 16, 20–24**). Klebs (reviewed by Whyte [**29**]) distinguished three stages in flower production: (*a*) development of the condition "ripeness-to-flower," (*b*) formation of floral primordia, and (*c*) development and expansion of floral structures. It is generally recognized that the succession of changes in flowering may be limited or inhibited at several stages and that a certain minimum vegetative growth must occur before "ripeness-to-flower" is reached and differentiation of floral primordia can take place. For many plants the extent of such minimum vegetative growth varies with environment; in others, floral initials are produced con-

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sistently after a certain amount of vegetative growth in spite of considerable environmental differences; and, in still others, the actual expression of flowering may be influenced by differential treatments either before or after attainment of the "ripeness-to-flower" state.

Perennial grasses favor investigations of these developmental changes, since many genetically identical growing points are available for study. These are presumably potentially able to flower but may be subjected individually to a range of internal and external environmental conditions. In grasses the transformation of a stem growing point from the vegetative to the reproductive state occurs abruptly. SHARMAN (**23**) considered this rapid change-over to indicate that some sort of minimum internal requirement must first be satisfied. He suggested using "number of leaves on the main stem as an index of maturity of the individual [culm] at any stage" of development, this number thus indicating the developmental state of the growing point before any outward signs of reproductivity are evident.

The use of this criterion in grasses is based on several studies, some of which suggest that it is dependent on, others that it is independent of, ecological relations. PURVIS (**20**) reported that, at a given temperature, variation in the amount of nitrogen supplied to winter rye had little effect on the minimal number of leaves produced before floral differentiation occurred. PURVIS and GREGORY (**21**) later, however, showed that this minimal leaf number was a variable, subject within limits to effects of vernalization or photoperiod. SHARMAN (**23**), using a pure strain of wheat, concluded that reduction in ability to photosynthesize carbohydrate by removal of leaf blades as they expanded did not appreciably influence the minimal leaf number, and NUTMAN (**16**) found that these numbers on seedlings developed from normal grains of rye weighing about 36 mg. each and from dwarf grains weighing only 2.3 mg. each were approximately the same.

SHARMAN (**24**), working with a perennial, couch grass (*Agropyron repens* [L.] Beauv.), found a minimal number of leaves formed on a tiller before it differentiated floral primordia; he postulated that this number had to be attained while the photoperiod was still favorable for floral initiation in that species or else no initiation would occur. RICE (**22**), investigating five species of native perennial range grasses in Oklahoma, reported that all but one, side-oats grama (*Bouteloua curtipendula* [Michx.] Torr.), gave "considerable evidence that any culm which does not have the number of expanded leaves specified for the species by a certain date does not initiate an inflorescence" but remains vegetative and that the number of leaves with expanded blades on a stem was "a fairly accurate index of the time of floral initiation in all species up to the end of the major period of inflorescence initiation for each species." In general, these findings indicate that in some grasses a correlation does exist between the number of expanded leaves present on a shoot and its stage of floral development.

In studies on smooth brome grass (*Bromus inermis* Leyss.) considerable variation in vegetative growth and reproductive activity of different clones has been reported (**4, 5, 7, 11, 12, 15, 26, 27, 30**). WALDRON (**27**), studying several thousand seedlings in North Dakota, noted that within a given clone the percentages of fertile tillers (those exserting flowering heads) and sterile tillers (those remaining externally vegetative) were relatively constant over a period of 3

years but that these percentages showed a wide range among different clones. He designated those clones with high percentages of fertile tillers as "ferticlinous" and those with high percentages of sterile tillers as "stericlinous" but also recognized the existence of a complete series of gradations among different clones. ALLARD and EVANS (**1**) reported that tillers of smooth brome grass failed to show internodal elongation and subsequent flowering when subjected only to short photoperiods. GALL (**9**) confirmed this and in addition also found marked irregularity in reproductive activity under certain photoperiods when using clonal sections of different sizes. He stated that these irregularities may have been the result of the failure of some of the growing points to reach the "ripe-to-flower" condition—by reason of age, position on plant, or other factors—prior to removal to the greenhouse and placing under photoperiodic treatment. He further stated that in this species the "attainment of the physiological condition resulting in the formation of floral initials as the first morphological sign of the reproductive state is with little doubt caused by a complex of factors." The factors which comprise this complex are not definitely known, but it would seem that a long photoperiod is not essential for the appearance of floral initials, since at Chicago under garden conditions they are first formed by early April during the year of flowering. GALL concluded, however, that long photoperiod is definitely related to normal development and elongation of culms and panicle initials and to the ripening of the fruit. SPRAGUE (**25**) reported that "1 hour of supplementary light during the middle of the normal winter night was almost as effective as the 6 hours at the end of the normal winter day [providing a 16-hour day] in initiating head formation on seedlings" of brome grass growing under greenhouse conditions.

GARDNER and LOOMIS (**10**), investigating thermal induction in brome grass, reported that no flowering occurred without exposure to low temperatures. They stated that "freezing is not required, and fewer than 14, perhaps no more than one, cold night is sufficient for induction." They also reported that, in addition to cool temperatures, short days are also required for induction. METCALFE (**13**), studying the interaction of several environmental factors on the induction and development of floral primordia of this species, reported no induction with plants grown under 18-hour photoperiods in spite of the temperature treatment applied. In contrast, GALL (**9**) stated that prechilling is not always a requirement for flowering in smooth brome grass, since he observed several clones in anthesis which had been maintained in a warm greenhouse continuously for over a year. Nevertheless, he found that plants with a period of winter chilling prior to removal to the greenhouse for study showed the most vigorous growth, flowering, and production of seed. SPRAGUE (**25**) found that brome seedlings started in the greenhouse in August were able to flower, given certain photoperiodic treatments, even though apparently never exposed to low winter temperatures. He stated that "these results, with plants started from seeds, are similar to those obtained previously with clones."

In no reported work with smooth brome grass has the possible relationship between reproductive activity and phases of growth and development in individual tillers been investigated. This study was therefore made to ascertain such possible correlations, especially relative to differences in the ecological cycle for tillers

initiated and growing at different seasons. Clones of widely diverse origin and type were studied in the Department of Botany experimental plots at the University of Chicago from October, 1949, through July, 1950, and from July, 1951, through March, 1952.

Material, methods, and environment

Cycle of growth and development (table 1)

Smooth brome grass has long been recognized as a cool-season grass—able to continue active growth late in the fall and to renew it relatively early in the spring. Minimum-maximum air temperatures recorded for different phases of the growth cycle are shown in table 1. The thermometer was installed among the plants under a protective shield which prevented direct exposure to the sun; the base of the mercury column was approximately 8.5 cm. above the soil surface.

At Chicago after mid-June, when most plants are in anthesis, tiller emergence becomes pronounced, whether or not flowering stems are harvested. New shoots continue to arise from both rhizome and basal axillary buds on into the fall of the year. Tillers which attain a certain size by midsummer show internodal elongation. These elongated[2] shoots continue to produce new leaves until they die back to the ground in late autumn and winter; and their basal axillary buds grow during late summer and early autumn. Tillers emerging in late summer and autumn do not show internodal elongation then but continue to initiate and exsert leaves until the cold fall weather and then stop growing. The number of exserted leaves on an unelongated shoot at this time is thus partly determined by its age since emergence. Upper leaves may still show considerable greenness through the end of November into early December, and even in early February some protected leaves expanded in the previous year may remain green. Expansion of additional leaves in late autumn is related to warm temperatures favorable for continued growth. Unelongated shoots remain relatively dormant from early December until mid-March, with their terminal growing points at or below the ground surface. Emergence of a few new tillers and appearance of new leaves on previously emerged, unelongated tillers may be evident by mid-March, when nightly temperatures are still below freezing; this new but less active period of tiller emergence extends to early May. Many newly emerging tillers represent shoots arrested in growth by cold winter temperatures while still below ground.

Floral initiation can be detected by dissection in early April, and it continues most of the month. These growing points are approximately at ground level. Inflorescence primordia appear first on shoots which emerged during the previous autumn and later on shoots emerging in the spring. Apparently cessation of floral initiation is correlated or coincidental with beginning of internodal elongation. In both fertile and sterile tillers the latter begins in late April, probably in response to increasing daylength (**9, 18, 25**). Inflorescences are first exserted after the third week in May, and exsertion extends approximately over a 2-week period. Anthesis begins after the first week in June and also lasts approximately 2 weeks. Seed ripens in late July, and the panicles turn brown.

Time intervals for the various stages should be recognized as approximate,

[2] An elongated tiller is defined as one in which the length of one (or more) internode is 2 mm. or more long.

since all clones studied did not exhibit them at precisely the same time. Chronological limits for these stages may be expected to vary for different strains, years, and geographical locations. For the clones studied, however, the variation observed at Chicago was not striking.

Specific observations

The ten clones studied critically represented a wide range of reproductive potentiality as indicated by data previously obtained by Olmsted (**18**) and by Gall (**9**), ranging from the "ferticlinous" to the "stericlinous" of Waldron (**27**). Five clones were highly fertile, two moderately fertile, and three relatively sterile (table 4). These clones had been retransplanted 5 feet apart in the garden in May, 1949, and were well established at the time of initial observation in October, 1949. They varied considerably, as is

TABLE 1

MINIMUM-MAXIMUM AIR TEMPERATURES (° F.) IN WEEK PRECEDING DATES GIVEN AND SEASONAL CYCLE OF BROME GRASS

Date	Temperatures		Activity*
	Min.	Max.	
Nov. 11, 1949...	32	74	
18.....	27	74	?
25.....	16	38	
Dec. 2.....	14	56	Cessation of growth in fall
9.....	12	47	?
15.....	6	61	
23.....	.....	.....	
Jan. 4, 1950...	4	60	
6.....	9	32	
13.....	14	52	
20.....	2	50	
27.....	9	67	Period of dormancy
Feb. 2.....	8	45	
9.....	12	44	
15.....	26	42	
25.....	2	39	
Mar. 4.....	2	42	
7.....	32	58	
16.....	10	57	Renewal of growth in spring
24.....	20	52	
31.....	22	63	
Apr. 7.....	26	58	First period of tiller emergence
14.....	20	72	Floral initiation
21.....	26	70	
28.....	28	82	
May 5.....	34	84	
12.....	39	86	
19.....	43	89	Internodal elongation
26.....	46	100	Peak of exsertion
June 1.....	46	92	
9.....	44	100	
15.....	52	92	Peak of anthesis
23.....	47	95	
30.....	54	96	Second period of tiller emergence
July 7.....	49	90	
14.....	54	98	Harvest (July 11–15)
Aug. 25.....	45	101	

* Time intervals indicated for various periods of activity are only approximate.

general for the species, in relative vigor, growth habit, and rate of spread.

In order to follow accurately the life-histories of tillers initiated and growing at different seasons, tillers were tagged at two different times so as to provide maximum variation in size and condition. In the period October 13–31, 1949, a hundred tillers were tagged in each clone (table 5). A numbered label was fastened to a tiller above its uppermost dead leaf blade and below the lowermost green blade; this position simplified subsequent leaf counts. A leaf blade was classified as dead when it was over 50% yellowed, darkened, or decayed.

Data recorded in October for each tagged tiller included: (*a*) position within the clump (center or periphery, and compass direction from center); (*b*) favorableness of position for growth at the first observation; (*c*) elongation, including any signs of basal internodal elongation; and (*d*) numbers of dead and living leaves. In the central and peripheral portions of the clones there were 248 and 752 tagged shoots, respectively (table 2). Numbers in eight equal radial segments of the peripheral portions of the clones, both individually and collectively, were approximately equal. The collective numbers of tagged shoots for each fell mostly between 90 and 100. Tiller position was estimated as favorable, moderately favorable, or unfavorable, based on light relations, degree of crowding, and position within the clump. The total number of shoots in each class was 459, 274, and 267, respectively. This grouping of tagged shoots was recorded for its bearing on their state at harvest—whether fertile, sterile, or dead (table 2).

During a second observation period, November 3–December 6, 1949, data on internodal elongation and leaf numbers were recorded for each tiller previously tagged (table 5).

Records were made in spring, 1950, of the first observed dates of initiation of inflorescence primordia, of internodal elongation, of exsertion of inflorescences, and of anthesis. Sufficient data on the duration of each activity were recorded to give an estimate of peak periods (table 1).

Onset of visible reproductive activity and of internodal elongation was determined by dissection of shoots from untagged clones at approximately weekly intervals starting shortly after growth renewal in the spring. Data recorded (table 6) per tiller included its year of emergence above ground, developmental state of growing point, any internodal elongation, and leaf counts.

In the spring and early summer newly emerged tillers were tagged for the ten critically studied clones on five different occasions; when tagged they were 3 inches or less in height and had only green leaves. No leaf-count data were taken except for group 4. The last group tagged consisted of shoots emerging from the ground during early summer.

Harvest for the ten clones was completed July 11–15, 1950. Each tagged tiller was dug out with minimal damage to the lowermost leaves and stem. Data recorded for each shoot included height, reproductive state, number of elongated internodes, and leaf counts (tables 2, 3). The height measured was the distance from immediately below the unelongated basal internodes of the stem to the tip of the panicle for fertile culms, and to the tip of the longest vertically outstretched leaf for sterile shoots. Leaf counts at harvest for shoots tagged in 1949 included number of dead leaves which had been produced, exserted, and died in 1949;

number of leaves dying in 1950; number of green leaves formed in 1950, including nonexserted ones in the case of sterile shoots; and total number of leaves produced during both 1949 and 1950. Data for new shoots tagged in 1950 included counts of leaves which came above ground in 1950; some leaves on these tillers, first appearing above ground in 1950, developed from primordia initiated during 1949. Decay of the lowermost leaves by harvest rendered an accurate count impossible of dead leaves on tillers emerging in 1949, so the total leaf number recorded at the second observation, fall, 1949, was taken as the total number of leaves exserted during 1949. Counts for the 1950 leaves were similarly complicated by such disintegration. For this reason total counts of leaves produced in 1950 are somewhat lower than might be expected in a comparison with the numbers of elongated internodes for individual tillers. The counts are still useful, however, in contrasting the different growth patterns of vegetative and reproductive shoots.

Data on numbers of elongated internodes (2 mm. or more) at harvest are accurate and most significant (tables 2, 3). To determine the uppermost elongated internode for sterile tillers, it was necessary to remove the upper leaves. For fertile culms the uppermost elongated internode counted was that one immediately below the reduced leaf subtending the panicle.

In order to determine whether tillers apparently sterile at harvest had initiated floral primordia which had been inhibited from developing, the growing points of ten untagged apparently sterile shoots for each of the ten clones were dissected. All these growing points were vegetative.

Results

Tillering

From October, 1949, to July, 1950, there was a general increase in basal area for the ten clones; in most it was so pronounced that the clumps, initially 5 feet apart, were at the time of harvest bordering immediately upon one another. Many studies (**2, 3, 7, 8, 14, 19**) have noted the so-called sod-bound condition for smooth brome grass. By summer, 1950, there were evidences of this condition in clones 5, 12, and 20, in which center shoots were dying out or growing much less vigorously than those of other clones. This corroborates PIPER's (**19**) report of marked decrease in yield of hay within sod-bound clumps.

There were two periods of active tiller emergence during 1950 (table 1). The first (table 6) began in mid-March (12½-hour daylength) prior to floral initiation and extended to early May (14½-hour daylength). The second, and more active, period started in mid-June (16-hour daylength) after the height of anthesis, and at the time of harvest in mid-July (15½-hour daylength) tillering was still continuing. Between these two main periods only a few scattered tillers emerged.

GALL (**9**) and WATKINS (**28**) both reported that the total number of tillers produced by smooth brome grass was greater on short than on long experimental constant photoperiods. During the present observations there was a general decrease in the rate of new tiller emergence in May and early June, when days were lengthening. However, this decrease was undoubtedly related to the series of complex physiological changes involved in floral initiation, internodal elongation, exsertion, anthesis, and fruit maturation.

In mid-June, at anthesis, renewal of

tiller emergence became evident. All newly emerged tillers were tagged June 21–27, a total of 423 for the ten clones. Earlier in the year less than 300 emerged in approximately 7 weeks. The spring period of tillering thus contributed considerably less than the summer and autumnal period to the total number of tillers produced for the year, especially since the second period extended to the end of the growing season. Also, many tillers emerging during the spring were arrested in development by cold weather while still below ground in the preceding fall and did not develop completely during early 1950 from rhizome or axillary buds.

Status of tagged tillers at harvest

Individual tillers were grouped according to developmental state at harvest in 1950 into four categories: fertile, sterile, dead, and injured. Of the 1000 tagged in October, 1949, there were 641, 137, 209, and 13, respectively, within these four classes. The number of dead tillers important for purposes of comparison with the other categories actually was 184 instead of 209, since this class included 25 shoots showing some internodal elongation when initially tagged. At that time it was expected that all such elongated shoots would die during the winter; this was confirmed. The injured class consisted of 13 shoots which were damaged before and at harvest in various ways so that reliable data could not be obtained from them. Of the remaining 962, the percentages of fertile, sterile, and dead, respectively, were 67, 14, and 19. The ranges in percentages for the ten clones were: fertile, 37–84; sterile, 1–38; and dead, 8–29.

A check was made to determine whether shoots tagged in the autumn for each clone were representative of that clone in proportion of fertile to sterile tillers at harvest (table 4). Counts were made of all living shoots elongated at harvest which had emerged during the autumn of 1949, both sterile and fertile. The comparison was not completely satisfactory, however, because of difficulty in distinguishing certain sterile shoots emergent in 1949 from those emergent in 1950. Nevertheless, percentages derived from the count of tagged shoots only are suggestive of the reproductive potentialities of clones. In other experiments Olmsted (**18**) secured data which are in general agreement with these estimates of reproductivity.

During spring, 1950, 252 newly emerged tillers were tagged, and at harvest 118 were dead, 66 fertile, 62 sterile, and 6 injured (table 4), or, respectively, 47, 26, 25, and 2%. The ranges of these percentages, omitting the injured group, among the different clones were: fertile, 4–62; sterile, 4–71; and dead, 21–83. These tillers, despite the high mortality, were located near clump peripheries where growth conditions were apparently favorable.

Height data at harvest in 1950 are shown in tables 2, 3, and 7. Fertile culms tagged in autumn, 1949, had the greatest mean height, 111.2 cm.; the other categories in order of diminishing size were: fertile culms emerging in spring of 1950, 75.4 cm.; sterile tillers emerging in autumn of 1949, 73.8 cm.; and sterile tillers emerging in spring of 1950, 66.4 cm. Height ranges within these groups were: fertile 1949, 30–172 cm.; fertile 1950, 36–142 cm.; sterile 1949, 12–110 cm.; and sterile 1950, 29–133 cm. Thus, at harvest, when fertile shoots were maturing and sterile shoots were still actively growing, the fertile shoots emerging in either year were generally taller than the sterile shoots, although the difference between the fertile 1950 and the sterile 1949

TABLE 2

TABLE 2

SUMMARY OF DATA FOR TILLERS TAGGED* IN 1949 ACCORDING TO STATUS AT HARVEST

Clone no.	No. of tillers						Stem data,† harvest 1950		Leaf Counts†										
		Location of tillers		Growth conditions					First observation autumn, 1949			Second observation autumn, 1949			Harvest 1950				
	Totals	Center	Periphery	Favorable	Moderately favorable	Unfavorable	No. elongated internodes	Height (cm.)	Dead	Green	Total	Dead	Green	Total	Dead 1949	Dead 1950	Green 1950	Total 1950	Total leaves
	Tillers fertile at harvest																		
14	73	15	58	48	13	12	8.9	117.4	0.9	4.4	5.3	1.8	4.7	6.5	6.5	2.9	5.5	8.3	14.9
7	36	2	34	20	14	2	8.0	101.7	2.7	5.2	7.8	4.4	4.3	8.7	8.7	5.1	3.3	8.4	17.1
6	82	18	64	51	16	15	8.2	91.4	1.7	5.6	7.3	3.5	4.6	8.1	8.1	3.7	4.3	8.0	16.1
2	60	9	51	21	19	20	8.6	121.1	1.8	5.5	7.3	3.4	4.5	7.9	7.9	3.7	4.0	7.8	15.7
4	35	3	32	22	9	4	9.4	112.2	1.4	4.9	6.4	3.3	3.7	7.0	7.0	4.6	6.3	10.9	17.9
23	76	15	61	65	10	1	9.8	138.7	1.3	5.8	7.1	3.5	3.9	7.4	7.4	4.8	5.8	10.6	18.0
16	62	7	55	26	26	10	7.8	87.5	2.2	5.3	7.5	4.3	3.6	7.9	7.9	4.2	3.2	7.4	15.4
5	66	7	59	33	23	10	8.9	119.9	2.0	6.0	8.0	4.8	3.6	8.4	8.4	3.5	4.8	8.3	16.7
12	73	17	56	40	15	18	8.1	94.8	2.0	5.7	7.8	5.4	2.7	8.1	8.1	4.4	3.4	7.9	16.0
20	78	7	71	38	31	9	7.2	122.7	2.2	5.2	7.5	4.2	3.7	7.9	7.9	3.1	3.6	6.7	14.6
Total or mean	641	100	541	364	176	101	8.5	111.2	.....	.....	.....	.....	.....	.....	7.8	3.9	4.4	8.3	16.1
	Tillers sterile at harvest																		
14	1	0	1	0	1	0	16.0	96.0	2.0	5.0	7.0	3.0	5.0	8.0	8.0	3.0	13.0	16.0	24.0
7	33	15	18	11	7	15	13.8	59.5	2.2	4.3	6.5	3.6	3.7	7.3	7.3	5.0	9.6	14.6	21.9
6	8	3	5	4	1	3	13.9	53.2	1.4	3.9	5.2	2.4	3.5	5.9	5.9	3.1	9.7	12.9	18.7
2	25	4	21	6	7	12	15.4	87.3	1.4	4.3	5.8	2.9	3.3	6.2	6.2	3.9	11.3	15.2	21.4

* Includes only tillers fertile, sterile, and dead at harvest and unelongated when tagged (does not include 25 tillers elongated when tagged or 13 tillers injured at harvest); no internodal elongation was observed for any of these tillers during autumn, 1949.

† Mean per tiller per clone.

TABLE 2—*Continued*

Clone no.	No. of tillers						Stem data,† harvest 1950		Leaf Counts†										
	Totals	Location of tillers		Growth conditions			No. elongated internodes	Height (cm.)	First observation autumn, 1949			Second observation autumn, 1949			Harvest 1950				
		Center	Periphery	Favorable	Moderately favorable	Unfavorable			Dead	Green	Total	Dead	Green	Total	Dead 1949	Dead 1950	Green 1950	Total 1950	Total leaves
	Tillers sterile at harvest—*Continued*																		
4	35	9	26	5	11	19	15.1	80.8	1.3	4.3	5.6	2.8	3.3	6.2	6.2	6.0	10.7	16.7	22.9
23	3	1	2	2	0	1	17.7	103.0	0.3	4.0	4.3	1.3	3.0	4.3	4.3	5.0	13.7	18.7	23.0
16	14	10	4	2	1	11	13.4	68.0	2.1	4.9	7.0	4.0	3.1	7.1	7.1	4.5	8.5	13.0	20.1
5	12	6	6	2	3	7	16.9	84.7	3.0	5.3	8.3	5.8	3.0	8.8	8.8	4.2	11.7	15.9	24.7
12	3	2	1	1	0	2	13.0	49.7	2.0	4.3	6.3	3.3	3.3	6.7	6.7	6.7	5.7	12.3	19.0
20	3	3	0	0	0	3	12.3	61.7	3.7	4.3	8.0	5.3	3.0	8.3	8.3	4.7	6.3	11.0	19.3
Total or mean	137	53	84	33	31	73	14.7	73.8	.....	.....	.....	.....	.....	.....	6.8	4.8	10.2	15.1	21.9
	Tillers dead at harvest, unelongated when tagged																		
14	14	8	6	4	3	7	.....	.....	1.1	4.3	5.4	4.1	1.8	6.0	.....	.....	.....	.....	.....
7	28	12	16	10	6	12	.....	.....	2.4	3.9	6.3	3.9	3.0	6.9	.....	.....	.....	.....	.....
6	8	4	4	4	0	4	.....	.....	1.5	4.0	5.5	2.9	2.9	5.7	.....	.....	.....	.....	.....
2	13	8	5	3	1	9	.....	.....	1.6	4.7	6.3	2.8	3.8	6.7	.....	.....	.....	.....	.....
4	22	13	9	0	7	15	.....	.....	2.2	4.5	6.7	4.1	2.9	7.1	.....	.....	.....	.....	.....
23	17	7	10	7	10	0	.....	.....	0.8	4.3	5.0	2.5	2.8	5.3	.....	.....	.....	.....	.....
16	21	5	16	9	6	6	.....	.....	3.0	4.6	7.6	5.2	2.7	7.9	.....	.....	.....	.....	.....
5	20	7	13	5	6	9	.....	.....	2.8	5.4	8.2	5.1	3.6	8.7	.....	.....	.....	.....	.....
12	23	11	12	5	7	11	.....	.....	2.5	4.2	6.6	4.9	2.3	7.2	.....	.....	.....	.....	.....
20	18	10	8	0	5	13	.....	.....	2.5	4.0	6.5	4.2	2.5	6.8	.....	.....	.....	.....	.....
Total or mean	184	85	99	47	51	86	.....	.....	.....	.....	.....	.....	.....	7.0	.....	.....	.....	.....	.....
Grand totals	962	238	724	444	258	260	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

shoots was not great. Tillers tagged in 1949 and 1950 which were fertile at harvest had greater mean heights than 1949 and 1950 tillers sterile at harvest, though the latter had larger mean numbers of elongated internodes (tables 2, 3). In many cases, however, after mid-July the sterile tillers eventually became taller than the fertile ones because of continued vegetative activity.

TABLE 3

MEANS OF HARVEST DATA FOR TILLERS TAGGED IN SPRING, 1950

Clone no.	Fertile tillers						Sterile tillers					
		Stem		Leaf counts				Stem		Leaf counts		
	No. of tillers	No. of elongated internodes	Height (cm.)	Dead	Green	Total	No. of tillers	No. of elongated internodes	Height (cm.)	Dead	Green	Total
14	4	8.0	109.0	3.0	4.5	7.5	3	14.3	77.3	2.0	12.0	14.0
7	1	7.0	102.0	5.0	2.0	7.0	4	14.5	58.2	3.2	12.5	15.7
6	17	6.9	64.8	3.4	2.8	6.2	6	12.7	55.7	2.7	9.3	12.0
2	1	7.0	100.0	3.0	4.0	7.0	15	13.3	64.7	2.9	10.6	13.5
4	1	7.0	57.0	2.0	4.0	6.0	10	11.7	57.8	2.9	9.7	12.6
23	4	9.0	128.7	4.2	5.5	9.7	12	19.0	99.2	3.6	15.1	18.7
16	21	6.6	63.8	3.5	2.7	6.2	5	11.4	46.2	3.4	8.8	12.2
5	3	6.7	83.0	3.0	2.7	5.7	1	13.0	43.0	4.0	9.0	13.0
12	5	7.0	64.0	3.4	3.6	7.0	3	11.0	48.0	5.7	6.7	12.3
20	9	6.9	84.1	3.7	2.7	6.3	3	11.7	54.7	4.7	7.7	12.3
Total	66	7.0	75.4	3.5	3.1	6.6	62	13.8	66.4	3.3	10.9	14.2

TABLE 4

NUMERICAL DISTRIBUTION INTO VARIOUS CATEGORIES AT HARVEST IN 1950 OF TILLERS TAGGED* IN BOTH 1949 AND 1950 AND OF ALL ELONGATED TILLERS EMERGED DURING BOTH 1949 AND 1950 LIVING AT HARVEST

Clone no.	Tillers tagged in 1949				Tillers tagged in 1950				Total no. elongated tillers emerged in 1949 and 1950			Estimate† of reproductivity
	Fertile	Sterile	Dead	Total	Fertile	Sterile	Dead	Total	Fertile	Sterile	Total	
14	73	1	14	88	4	3	23	30	149	43	192	Highly fertile
7	36	33	28	97	1	4	15	20	58	140	198	Relatively sterile
6	82	8	8	98	17	6	8	31	230	108	338	Highly fertile
2	60	25	13	98	1	15	10	26	161	306	467	Relatively sterile
4	35	35	22	92	1	10	3	14	72	222	294	Relatively sterile
23	76	3	17	96	4	12	13	29	106	77	183	Highly fertile
16	62	14	21	97	21	5	8	34	158	159	317	Moderately fertile
5	66	12	20	98	3	1	19	23	198	159	357	Moderately fertile
12	73	3	23	99	5	3	11	19	191	87	278	Highly fertile
20	78	3	18	99	9	3	8	20	296	133	429	Highly fertile
Total	641	137	184	962	66	62	118	246	1619	1434	3053	

* Includes only tillers unelongated when tagged.

† Evaluation based mainly on distribution at harvest of tillers tagged in 1949.

INTERNODAL ELONGATION

Of the 1000 shoots tagged during the fall of 1949, 975 showed no internodal elongation when tagged. Elongation of comparable fertile and sterile shoots on three other clones was first generally observed by dissection on May 9, 1950 (15-hour daylength), although as early as April 28 slight elongation was detected for one shoot from a different clone. Since GALL (**9**), working with some clones used in this study, noted no significant variation among them in time of occurrence of different phases of growth and development, it may be assumed that the tagged shoots also began internodal elongation in early May.

Shoots from both spring and early summer periods of emergence in 1950 exhibited internodal elongation at harvest; however, shoots emerging shortly after mid-July ($15\frac{1}{2}$-hour daylength) did not elongate in the same year, presumably because the shortening daylengths became unfavorable for elongation before they developed sufficiently to receive the effective stimulus. Several investigators (**1, 9, 18, 25**) have found that short constant photoperiods are unfavorable for internodal elongation in smooth brome grass.

At harvest in 1950 the mean numbers of elongated internodes for all fertile and sterile shoots initially tagged in 1949 were 8.5 and 14.7, respectively (table 2). For the ten clones the range of mean numbers for this fertile class was from 7.2 (clone 20) to 9.8 (clone 23); for sterile shoots, from 12.3 (clone 20) to 17.7 (clone 23). The range in actual numbers for shoots tagged in 1949 was 5–12 elongated internodes for fertile culms and 8–20 for sterile shoots. The ranges are substantially narrower, however, when the extremes are excluded. For the fertile shoots, 96.7% fell within the range of 7–10 elongated internodes and 66.6% within the range of 8–9; for the sterile, 93.4% fell within the range of 11–18 and 61.3% within the range of 14–16. Often individuals exhibiting extremes of the ranges also tended to be atypical of their respective classes in other ways. For instance, a sterile shoot from clone 7 which had only 8 elongated internodes was spindly and weak. Similarly, a shoot with only 5 elongated internodes from clone 12 had poorly developed panicles.

The mean numbers of elongated internodes at harvest for shoots emerging and tagged in 1950 were 7.0 and 13.8, respectively, for fertile and sterile shoots (table 3). For the ten clones the range in means for fertile tillers was 6.6 (clone 16) to 9.0 (clone 23); for sterile shoots, 11.0 (clone 12) to 19.0 (clone 23). The total range for fertile culms was 6–9; for sterile tillers, 7–22. By excluding extremes, somewhat smaller ranges were obtained; however, the clustering about the mean was not so pronounced for shoots emerging and tagged in 1950 as for those emerging and tagged in 1949. Thus, for fertile culms tagged in 1950, 92.5% fell within the range of 6–8 elongated internodes and 73.1% within the range of 6–7; for sterile shoots, 95.1% fell within the range of 10–21 and 67.7% within the range of 11–15.

In general, tillers emerged and tagged in spring, 1950, were less vigorous and had smaller elongated internode and leaf numbers than those emerging in autumn, 1949; the former had had less time for growth, since they emerged at varying times after growth conditions had again become favorable in the spring. Thus, sterile shoots emerging during spring, 1950, although still producing new leaves at harvest, had not attained so high a mean for total leaf number or total number of elongated internodes during 1950

as had sterile shoots which emerged during autumn, 1949. The smaller mean numbers of elongated internodes and of leaves produced during 1950 for the fertile culms emerging in 1950, as compared with the sterile ones or with fertile ones emerging in 1949, are a result of transformation of growing points from the vegetative to the reproductive state at a time when this was generally occurring; the new 1950 shoots underwent this greater than the mean total number produced during 1950 by the fertile ones (15.1 and 8.3, respectively). Since the mean number of leaves exserted during 1949 was somewhat lower (6.8) for shoots which remained sterile than for fertile culms (7.8), the larger total number of leaves for shoots sterile at harvest is definitely associated with the longer period of active leaf initiation experienced by the latter shoots during 1950.

TABLE 5

CHANGES IN MEAN NUMBERS OF DEAD, GREEN, AND TOTAL LEAVES BETWEEN TWO PERIODS OF OBSERVATION IN AUTUMN, 1949, FOR TILLERS FERTILE, STERILE, AND DEAD AT HARVEST

Clone no.	Dates of observations		No. of days between observations	Status of tiller at harvest											
				Fertile				Sterile				Dead*			
	First	Second		No. of tillers	Dead leaves	Green leaves	Total leaves	No. of tillers	Dead leaves	Green leaves	Total leaves	No. of tillers	Dead leaves	Green leaves	Total leaves
14	10–13	11–3	21	73	+0.9	+0.3	+1.2	1	+1.0	0.0	+1.0	14	+3.0	−2.4	+0.6
7	10–14	11–7	24	36	+1.7	−0.9	+0.9	33	+1.4	−0.6	+0.8	28	+1.5	−0.9	+0.6
6	10–18	11–8	21	82	+1.7	−1.0	+0.8	8	+1.0	−0.4	+0.6	8	+1.4	−1.1	+0.2
2	10–20	11–11	23	60	+1.6	−1.0	+0.6	25	+1.4	−1.0	+0.4	13	+1.2	−0.8	+0.4
4	10–21	11–14	25	35	+1.8	−1.2	+0.6	35	+1.5	−1.0	+0.5	22	+1.9	−1.5	+0.4
23	10–24	11–15	23	76	+2.2	−1.9	+0.3	3	+1.0	−1.0	0.0	17	+1.7	−1.5	+0.2
16	10–26	11–16	22	62	+2.1	−1.7	+0.4	14	+1.9	−1.8	+0.1	21	+2.1	−1.9	+0.3
5	10–27	11–18	23	66	+2.7	−2.4	+0.4	12	+2.8	−2.3	+0.5	20	+2.3	−1.8	+0.5
12	10–28	11–30	34	73	+3.3	−3.0	+0.3	3	+1.3	−1.0	+0.3	23	+2.4	−1.8	+0.6
20	10–31	12–6	37	78	+2.0	−1.5	+0.5	3	+1.7	−1.3	+0.3	18	+1.7	−1.4	+0.3

* Includes only dead tillers unelongated when tagged.

transformation with relatively few leaves and ultimately elongated only a few internodes. The possibility should be noted that many fertile shoots which emerged in 1950 had poorly developed panicles as a consequence of the low number of leaves able to contribute to their development.

Leaf counts

At harvest the mean total leaf number of the ten clones for all fertile culms tagged in 1949 was 16.1; for sterile shoots, 21.9 (table 2). Sterile shoots were still producing new leaves when harvested. Similarly, at harvest the mean total number of leaves produced during 1950 by sterile shoots tagged in 1949 was

Leaf counts in 1949.—In the 1949 data (tables 2,5) for tagged shoots which were fertile, sterile, or dead at harvest in 1950, variation in leaf number for any one count within the various classes was consistently wide. Even by excluding extremes of ranges and by considering differing percentages of the totals for any one category, the ranges were still large and overlapping. Thus, for smooth brome grass the leaf number formed on a tiller at any given time in the autumn is only one of the factors operative in determining its fate the following year. However, the mean number of leaves (6.8) exserted during 1949 by tillers remaining sterile in 1950 for all clones was lower than this

number (7.8) for fertile ones. Individual clones generally exhibited the same trend if representative numbers were included in the counts. Similarly, the mean number of leaves (5.8) exserted in 1949 by fertile shoots from other clones dissected before harvest in 1950 was also larger than this mean number (4.0) for vegetative shoots. Exceptions to this trend were noted for clones 5, 14, and 20; but here the numbers of sterile tillers available for deriving the means were too few to give the larger mean number observed for the sterile tillers much significance. For clones 2, 4, and 7, all relatively sterile, the mean total leaf number exserted in 1949 by shoots fertile in 1950 was consistently greater than this number on shoots sterile in 1950. But, while in these particular clones the total number of leaves exserted by a tiller in late 1949 was apparently closely correlated with its final reproductive state in 1950, the ranges of variation in 1949 leaf counts for all shoots fertile at harvest indicate that this close correlation is not general. Other factors, such as relative favorableness of growth conditions for individual shoots and reproductive potentialities of clones, must be considered in accounting for fertility or sterility.

Other trends in 1949 leaf counts for tillers fertile and sterile in 1950 were observed at both first and second periods of observations (tables 2, 5). Mean number of green leaves per tiller was greater than mean number of dead leaves for all clones until mid-November. Then, after the first severely cold weather, the mean number of dead leaves per tiller became the greater.

Comparison on a clonal basis of the mean total number of leaves in the autumn on tagged unelongated shoots dead at harvest with these numbers for fertile and sterile shoots revealed no consistent pattern (table 2). Relatively unfavorable growth conditions were largely responsible for death of shoots irrespective of number of leaves present. Numbers of unelongated dead shoots within favorable, moderately favorable, and unfavorable categories of growth conditions were 47, 51, and 86, respectively (table 2). Thus, there was a definite relationship between conditions for growth, as estimated in autumn, and survival. Approximately half the total number of tagged, unelongated dead shoots (85 out of 184) were located in centers of clumps where growth conditions were generally unfavorable (table 2).

Shoots dead at harvest had shown a tendency to die early. All ten clones for the first period of observation in 1949 (table 2) showed a greater number of green than of dead leaves per tagged tiller regardless of whether it was fertile, sterile, or dead at harvest in 1950. At the second observation in 1949, however, shoots dead at harvest generally showed more leaf death than did fertile or sterile shoots, even prior to the advent of nightly subfreezing, extreme temperatures. Another indication of waning vigor in 1949 of the dead-shoot category was shown by comparing relative increase in mean total leaf numbers between the first and second periods of observation (table 5). Generally shoots fertile at harvest then showed the greatest increase in leaf number, sterile shoots the next greatest, and dead shoots the least. Shoot death was probably related to a general retrogression in vigor which started early in the fall; affected shoots were consequently less able to withstand sudden cold. Within a week there was a temperature drop from 74° F. to 16° F.

LEAF COUNTS IN 1950.—Shoots emerging in spring, 1950, also exhibited much variation in leaf numbers at harvest

(tables 3, 7). Mean leaf numbers for these tagged shoots, including all clones, were 6.6 and 14.2, respectively, for fertile and sterile shoots. Ranges were 5–11 for fertile culms and 7–22 for sterile tillers. Their ranges in mean leaf counts per clone were 5.7 (clone 5) to 9.7 (clone 23) for fertile, and 12.0 (clone 6) to 18.7 (clone 23) for sterile, shoots. For the fertile culms 72.7% had 6–7 leaves, and for the sterile shoots 62.9% had 12–15 leaves. Considerably fewer total leaves were produced by fertile and sterile shoots emerged and tagged in spring, 1950, than by shoots emerged and tagged in autumn, 1949 (tables 2, 3). The numbers of leaves expanding during 1950 on fertile shoots tagged in 1949 and 1950 more nearly correspond than do the total numbers produced; the same is true of sterile shoots. Even so, the shoots emerging in 1950 produced in that spring fewer total leaves per tiller than did the tagged 1949 shoots. The former had a shorter period available actively to initiate leaves, since they emerged at varying times after the 1949 shoots had actively resumed growth. Difference in total number of leaves for 1950 tillers fertile and sterile at harvest was related to the fact that after floral initiation no further increase in leaf number was possible for fertile culms.

The 1950 shoots tagged late in the spring period of tiller emergence tended to remain vegetative or, in some clones, to die before harvest. These vegetative shoots apparently had insufficient time to initiate the number of leaves indicative of the "ripe-to-flower"condition before close of the active period of floral initiation. For the dead shoots, there may have been some inhibition of further growth related to the simultaneous floral development in other tillers.

Additional leaf-count data were obtained in the spring of 1950 at the time of floral initiation by dissecting 127 tillers which emerged in both 1949 and 1950 from untagged clones. At approximately weekly intervals a record was made of the numbers of dead leaves, of green leaves partly or completely exserted, of nonexserted green leaves, including all distinguishable leaf primordia, of total leaves, of elongated internodes, and of the state of the growing point for each dissected tiller. Of these tillers, 57 were reproductive and 70 were vegetative when dissected (table 6).

At the initial dissection on March 21, 1950, all shoots emerging in autumn, 1949, were still vegetative, and new leaves were just starting to protrude beyond the sheaths of the dead ones. Floral initiation was first observed on April 5 for shoots emerging in 1949; on April 27, for shoots emerging in 1950. Internodal elongation was first detected on April 28 for one vegetative shoot. All shoots dissected thereafter, except one on May 9, showed elongation. At every preharvest dissection up to the time of internodal elongation the mean number of leaves was greater for reproductive shoots emerging in 1949 than for vegetative tillers (table 6). At subsequent dissections the mean number was larger for sterile shoots; this difference increased until harvest in July, since the fertile shoots initiated no leaves after floral initiation, while the sterile ones were still forming them at harvest. The mean number per culm of dead leaves which had been exserted in 1949 was greater at every dissection for fertile than for sterile tillers, confirming similar observations for the ten tagged clones. The mean number of green leaves with blades at least partly exserted was slightly larger for the fertile shoots at every preharvest dissection except the last, when continuing initiation

TABLE 6

PREHARVEST DISSECTION DATA IN 1950 FROM UNTAGGED CLONES

Sampling dates	No. of tillers		No. dead leaves				No. green leaves exserted				No. green leaves not exserted				Total no. green leaves				Total no. leaves			
			Range		Mean		Range		Mean		Range		Mean		Range		Mean		Range		Mean	
	R*	V†	R	V	R	V	R	V	R	V	R	V	R	V	R	V	R	V	R	V	R	V
	Data for shoots emerging in 1949																					
Mar. 21, 24	0	12	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	11–17	.....	13.3
Apr. 5, 6	1	3	.....	5–8	.....	6.7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	15	13–15	15.0	14.0
11, 12	9	6	4–11	2–6	6.4	4.0	1–4	2–3	2.7	2.3	5–8	5–8	7.0	6.3	9–11	7–10	9.7	8.7	14–21	9–16	16.1	12.7
20, 21	10	5	3–7	2–5	4.5	3.4	3–4	2–3	3.8	2.8	5–8	6–7	6.5	6.8	9–11	9–10	10.3	9.6	14–17	11–15	14.8	13.0
27	8	1	5–8	3	6.1	3.0	4–5	4	4.2	4.0	5–6	6	5.6	6.0	9–10	10	9.9	10.0	15–18	13	16.0	13.0
May 9‡	7	0	4–10	.....	6.8	.....	4–5	.....	4.7	.....	3–5	.....	4.0	.....	8–10	.....	8.7	.....	12–19	.....	15.6	.....
17	3	10	4–9	2–6	6.7	4.0	6–7	3–6	6.7	5.1	1–2	5–8	1.7	6.2	8–9	8–13	8.3	11.3	12–17	13–19	15.0	15.3
25	4	5	4–7	2–4	5.7	3.4	5–7	5–7	5.7	6.0	0–2	6–8	1.5	7.0	7–8	12–14	7.2	13.0	11–14	15–18	13.0	16.4
Total	42	42	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	7–11	.....	9.4	.....	11–21	.....	15.3	.....
	Data for shoots emerging in 1950																					
Apr. 11, 12	0	7	.....	0	.....	0.0	.....	1–3	.....	2.0	.....	5–6	.....	5.3	.....	6–9	.....	7.3	.....	6–9	.....	7.3
20, 21	0	10	.....	0–1	.....	0.3	.....	2–4	.....	3.3	.....	5–7	.....	5.9	.....	8–11	.....	9.2	.....	8–12	.....	9.5
27, 28§	10	4	0–2	0	0.7	0.0	3–4	4–5	3.7	4.2	4–8	6–7	6.3	6.5	8–11	10–11	10.0	10.7	8–12	10–11	10.7	10.7
May 9‡	4	3	1–2	1–2	1.2	1.3	3–5	4	4.0	4.0	4–6	5–7	4.7	6.0	8–9	9–11	8.7	10.0	9–11	10–12	10.0	11.3
17	0	1	.....	2	.....	2.0	.....	5	.....	5.0	.....	6	.....	6.0	.....	11	.....	11.0	.....	13	.....	13.0
25	1	3	3	1–4	3.0	3.0	5	5–6	5.0	5.3	2	6–7	2.0	6.3	7	11–12	7.0	11.7	10	13–16	10.0	14.7
Total	15	28	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	8–12	.....	10.5	.....

* Tillers which had initiated floral primordia.

† Tillers which were vegetative; it should be remembered that some tillers included in the vegetative group up through April 28 might have eventually become reproductive, since floral initiation occurred until around the end of April.

‡ One vegetative tiller with ten leaves from a rhizome bud showed no internodal elongation; all other tillers were elongated at this and future sampling dates.

§ On this date one vegetative tiller with eleven leaves showed slight internodal elongation; all others were unelongated at this and preceding sampling dates.

of new leaves by sterile shoots resulted in their finally exceeding the fertile ones.

Data for shoots emerging in 1950 were lacking or spotty for many preharvest dissections; hence, trends are not clear (table 6). Contrast in mean leaf number between fertile and sterile shoots was not striking in most cases where data were available; this lack of contrast may indicate that some vegetative tillers were on the verge of becoming visibly reproductive. Probably the most significant data are those for mean leaf number for each dissection. This increased markedly for each successive dissection up to the time of internodal elongation. For sterile shoots this increase was continued thereafter, but for fertile tillers the number remained relatively constant in subsequent dissections. This is interpreted to mean that shoots emerging in 1950 and eventually becoming reproductive reached the necessary developmental state for floral initiation—as indicated by the increased total number of leaves—prior to internodal elongation.

It is of interest to compare data obtained by these periodic preharvest dissections with those of final harvest of the fertile shoots which emerged in both 1949 and 1950 (table 7). The mean total numbers of leaves were: preharvest dissected shoots emerging in 1949, 15.3; harvested shoots emerging in 1949, 16.1; preharvest dissected shoots emerging in 1950, 10.5; and harvested shoots emerging in 1950, 6.6. Mean total numbers of leaves for preharvest dissected and harvested tillers emerging in 1949 are nearly the same. Total mean numbers of leaves for preharvest and harvested shoots emerging in 1950 are different, but the significance of this difference is problematical. The mean for preharvest shoots is based on only 15 shoots, and it is difficult accurately to determine leaf numbers for culms at harvest because of the disintegration of the lowermost leaves. Recorded mean numbers at harvest for fertile shoots emerging in 1950 were undoubtedly somewhat lower than the actual values.

The mean numbers of green leaves present in 1950 on fertile tillers were: preharvest dissected 1950 shoots, 10.5; preharvest dissected 1949 shoots, 9.4; harvested 1949 shoots, 8.3; harvested 1950 shoots, 6.6 (table 7). Within the various categories ranges of like magnitude were generally observed. It is assumed that physiological requirements for transformation of growing points from the vegetative to the reproductive state were similar for all these fertile shoots. It is also possible that the number of green leaves present during the year of actual flowering may partially determine reproductivity. In the spring it may be necessary for sufficient additional leaves to be initiated before conversion to the reproductive state can occur. The data suggest that a minimum but variable number of leaves is formed before floral primordia are initiated. This requirement could be in addition to other factors involved in the attainment of "ripeness-to-flower," and the variability of this number indicates that still other factors are important. Nevertheless, the data definitely show that a variable minimal number (about 5–14) of green leaves is present during the year of actual flowering before floral initiation occurs and that this number is present before the end of the period during which floral primordia are formed (table 8). By excluding different numbers of tillers which deviate from the mean, the range of this minimal green-leaf number is defined more sharply: 94%, 6–11; 88% 6–10; 78%, 7–10; 68%, 7–9; and 51%, 7–8.

At harvest many fertile culms, emerged

TABLE 7

COMPARISON OF STEM AND LEAF COUNT DATA FOR TILLERS FERTILE OR STERILE IN 1950 AND EMERGING IN 1949 OR 1950

Tiller classes	Year of emergence	No. of tillers	Stem data						Leaf-count data								
			Height of tillers			No. of elongated internodes			Total leaves expanded 1949			Total leaves expanded 1950			Total leaves from 1949 and 1950		
			Mean	Range*	Range†	Mean	Range*	Range†	Mean	Range*	Range†	Mean	Range*	Range†	Mean	Range*	Range†
Fertile:																	
Tagged	1949	641	111.2	30–172	87.5–138.7	8.5	5–12	7.2–9.8	7.8	2–15	6.5–8.7	8.3	5–14	6.7–10.9	16.1	8–24	14.6–18.0
Preharvest dissected	1949	42	.....	.....	.....	.....	.....	.....	5.8	3–11	.....	9.4	7–11	.....	15.3	11–21	.....
Tagged	1950	66	75.4	36–142	57.0–128.7	7.0	6–9	6.6–9.0	.....	.....	.....	6.6	5–11	5.7–9.7	.....	.....	.....
Preharvest dissected	1950	15	.....	.....	.....	.....	.....	.....	.....	.....	.....	10.5	8–12	.....	.....	.....	.....
Sterile:																	
Tagged	1949	137	73.8	12–110	49.7–103.0	14.7	8–20	12.3–17.7	6.8	3–12	4.3–8.8	15.1	8–20	11.0–18.7	21.9	13–29	18.7–24.7
Preharvest dissected	1949	42	.....	.....	.....	.....	.....	.....	4.0	2–8	.....	.....	.....	.....	.....	.....	.....
Tagged	1950	62	66.4	29–133	43.0–99.2	13.8	7–22	11.0–19.0	.....	.....	.....	14.2	7–22	12.0–18.7	.....	.....	.....
Preharvest dissected	1950	28	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

* Range of actual numbers for ten clones.

† Range of mean numbers for ten clones.

in 1950 and with low total numbers of leaves, were decidedly spindly and only weakly reproductive, a condition possibly related to the low leaf number. A low number of leaves may limit the ability of the shoots to supply adequately what is needed for normal development at the growing points—"ripeness-to-flower" may not have been fully attained.

It seems that those shoots which attained "ripeness-to-flower" prior to internodal elongation became reproductive, whereas shoots which began elongation while still vegetative did not undergo such transformation. The larger mean leaf number present before internodal elongation for shoots which became fertile might well be important in their attainment of the internal state necessary for reproduction. However, apparently no one leaf number can serve as a critical measure of this attainment; the data revealed definite ranges in total leaf number for both 1949 and 1950 fertile and sterile shoots, and these overlapped one another. Those shoots with the greater leaf number before internodal elongation apparently most often achieved "ripeness-to-flower"; but, since this was not always true, other factors must also play important roles in reproductivity.

TABLE 8

RANGES IN NUMBERS OF GREEN LEAVES PER TILLER EXPANDED DURING 1950 BY FERTILE TILLERS EMERGING IN 1949 AND 1950 AS SHOWN BY INCLUDING DIFFERENT PROPORTIONS OF TILLERS

Tiller classes	Year of emergence	No. of tillers	Range*	%†	No. of tillers	Range*	%†	No. of tillers	Range*	%†	No. of tillers	Range*	%†
Preharvest dissection	1949	42	7–11	100	38	8–11	90	31	8–10	74	25	9–10	59
Tagged	1950	641	5–14	100	634	6–12	99	526	7–10	82	471	7–9	73
Preharvest dissection	1950	15	8–12	100	13	10–12	87	9	10–11	60	7	10	47
Tagged	1950	66	5–11	100	64	5–9	97	57	5–7	86	48	6–7	73
All tillers		764	5–14	100	670	6–10	88	599	7–10	78	522	7–9	68

* Range of actual numbers for individual tillers.
† Percentage of tillers included in range.

Floral development

In this paper, following the usage of RICE (**22**), " 'floral development' refers to that part of the reproductive cycle beginning with 'floral initiation' (initiation of the inflorescence primordia), and progressing through 'exsertion' (appearance of the inflorescence above the surrounding leaf sheath) and 'flowering' (anthesis)." GALL (**9**), working with some of the same clones used in this study, reported that floral primordia in the Chicago area were first observed by dissection of various clones April 8–14, 1947. In every case he first found floral primordia on shoots with leaves 20 cm. or more long and with unelongated stems. These tillers must have emerged during the fall of 1946, since insufficient time had elapsed for them to attain that size in the spring of 1947. During the present study floral primordia were first detected on one tiller of an untagged clone on April 5, 1950; and on April 11 four of five clones dissected

showed primordia. Floral initiation in the ten tagged clones undoubtedly occurred during approximately this same period.

On shoots emerging during 1950 floral primordia were first found on April 27 on three clones. One vegetative shoot showed slight internodal elongation on April 28, and by May 9 elongation was general for all clones dissected. Starting on April 27 the length of dissected developing inflorescences was measured. Sizes of inflorescence primordia dissected on May 9, and thereafter, made it evident that they had been initiated some time earlier. It is suggested that floral initiation had probably ceased around the end of April, coincidental with the beginning of internodal elongation. Thus the period of active floral initiation was approximately 3 weeks long (April 5–27). Investigations with other grasses have indicated that elongation may or may not be related to the reproductive process (**9, 17, 20, 22**).

A few inflorescences were exserted on each of the ten clones on May 25. Two weeks later 96% of the tagged fertile shoots on one clone had exserted inflorescences. On other clones 93–100% of the inflorescences on tagged culms were exserted within 3 weeks after the initial observation. The peak of exsertion thus fell within the first 2 weeks following the first observed exsertion.

Anthesis was first detected on June 9 on an untagged clone; six of the ten tagged clones exhibited it as of June 12, nine by June 14, and the remaining clone, no. 4, on June 18. All ten clones, except for clone 4, were no longer in anthesis on June 27, and clone 4 not after July 3. Anthesis thus covered approximately a 2-week period for each clone regardless of when first observed. At the time of harvest the grains were ripening as indicated by general drying-out and browning of the panicles.

Discussion

The data obtained have been examined to see whether some relationship does exist between the leaf number present on a tiller in smooth brome grass and the developmental state of its growing point. SHARMAN postulated (**23**) that the leaf number initiated is a possible measure of the extent to which the inner physiological state necessary for subsequent morphological expression of reproductivity has been attained. Before discussing this further, other pertinent factors defined in this study must also be considered.

The relative fertility or sterility of a clone of brome grass is in part genetically determined. Nevertheless, this investigation has shown that the position of the individual tillers within the clump and the associated favorableness of growth conditions are apparently significant, especially for those tending to be sterile. Of the 724 peripheral shoots unelongated when tagged in the autumn of 1949, at harvest 541 were fertile, 99 dead, and 84 sterile (table 2). The clump periphery, therefore, provided favorable conditions for fertility, though no particular portion was most favorable. Of the tagged centrally located tillers, 100 were fertile, 85 dead, and 53 sterile, indicating conditions decidedly less favorable for fertility. Data on favorableness of growth conditions as estimated from tiller position in the clump, light relations, and degree of crowding indicated close correlation with status of individual tillers at harvest. Of tillers which were unelongated when tagged and then grown under rather favorable conditions, 364 were fertile, 47 dead, and 33 sterile at harvest. Under moderately favorable growth con-

ditions, 176 shoots were fertile, 51 dead, and 31 sterile. Tillers growing under unfavorable conditions showed 101 fertile, 86 dead, and 73 sterile. Comparison of distribution of tillers within these three categories of favorableness separately for fertile and sterile shoots further emphasizes importance of growth conditions. For example, 57% of the 641 shoots fertile at harvest had favorable growth conditions, 27% moderately favorable, and 16% unfavorable. But of 137 shoots sterile at harvest, 24% had favorable growth conditions, 23% moderately favorable, and 53% unfavorable.

Data on individual clones make it clear, however, that other factors must be considered. The relative favorableness of growth conditions for individual tillers was not significantly important for highly fertile clones, since only a few of the tagged shoots alive at harvest remained vegetative. Apparently for these clones the tendency to flower was so pronounced that the individual shoots became reproductive even under unfavorable growth conditions. For relatively sterile clones, however, growth conditions for tillers were apparently important in conjunction with still another factor, leaf number, in the reproductivity of individual tillers. For the three relatively sterile clones (2, 4, and 7), unelongated shoots with the greatest number of leaves exserted in the autumn most often initiated floral primordia the following spring. However, a considerable range in autumnal leaf number allowed reproduction. In many cases shoots with low numbers of leaves in the fall were still able to become reproductive providing they had grown under rather favorable conditions. This suggests that, in general, shoots with large numbers of leaves, as well as those with small numbers but growing under favorable conditions, were able by the close of the growing season in the autumn to reach that state of development necessary for future floral initiation.

As indicated in the Introduction, the extent to which winter chilling influences development of "ripeness-to-flower" has not been decisively clarified for smooth brome grass. Though the ten clones were all exposed to similar low winter air temperatures, growing points of individual tillers were not necessarily uniformly chilled because of differences in their positions relative to ground surface, in their positions within the clump, and in the protection afforded by inclosing leaf sheaths and by snow cover during certain periods. Although any chilling requirement had probably been satisfied for individual tillers, microclimatic differences were undoubtedly present and of some possible significance in reproductivity.

The dissection data obtained for shoots emerging during both 1949 and 1950 suggested that a minimum number of green leaves, including primordia, are present during the year of flowering prior to internodal elongation before floral primordia are initiated. SHARMAN (**24**), investigating couch grass (*Agropyron repens* [L.] Beauv.), reported that "counting the transition leaf as the first, the main axis usually produces ten to eleven green leaves in all" before becoming reproductive and that "observations spread over four years indicate that while there may be some slight variation, in this latitude at least, about 80 per cent or more of the main shoots conform to these numbers." The data for smooth brome grass reveal similar conformity to a lesser extent in the number of green leaves present on fertile culms during the year of flowering. By considering successively fewer fertile tillers of the total population, there is a retrogression in range of

green-leaf number (table 8); slightly less than 80% of all fertile tillers fell within the range of 7–10. Thus, for smooth brome grass more variation is found in the number of green leaves present on individual tillers during the year of flowering before floral initiation than for couch grass; nevertheless, the trend is similar. The diversity in origin and type of the clones used in this study is undoubtedly an important factor in accounting for the observed variability in smooth brome grass, whereas the couch grass plants that SHARMAN studied were practically all from one "neighborhood" (**27, 30**).

Data from this study strongly suggest also a relationship between internodal elongation and reproductivity for individual tillers. Other investigations with different grasses have indicated that elongation may or may not be related to the reproductive process. PURVIS (**20**) recognized that stem growth in cereals is evidently correlated in some way with floral development and stated that, in some cases where floral initiation failed, there was then no internodal elongation. In still other instances elongation did not begin until after the initiation of floral primordia in the spring, and only those internodes which were between green leaves showed elongation. Some other grasses normally elongate their stems prior to floral initiation. OLMSTED (**17**), working with a strain of side-oats grama, stated that internodal elongation occurred prior to floral initiation and "was neither a sign nor an effect of reproductive activity in this strain." RICE (**22**), however, studying another strain of the same species, reported that it showed practically no internodal elongation prior to floral initiation and also stated that, unless floral primordia were formed, almost no elongation was observed even as late as October. It would thus seem that internodal elongation, at least for some species or strains, is in some way related to reproductive activity.

WATKINS (**28**) stated that shading resulted in greater numbers of elongated internodes on fertile shoots of brome grass and more uniform lengths of internodes. He did not specify the number of internodes produced under full light or the extent of increase under shade. Thus a fertile culm with more elongated internodes than the mean number for its clone may conceivably be an expression of this effect, in part, at least, brought about by shading in crowded portions of the clumps. In some cases fertile shoots with maximum numbers of elongated internodes had more than the usual number of elongated basal internodes less than 1 cm. long.

GALL (**9**), working with seven clones of smooth brome grass transplanted into the greenhouse on April 14 and placed under a 13-hour photoperiod, reported that after 6 weeks two clones exhibited a few fertile culms which had about six elongated internodes each. The divisions had been moved to the greenhouse after floral initiation was assumed to have occurred but prior to culm elongation. By June 3 the panicles on one clone were well developed and nearing exsertion but were somewhat less so on the other clone. However, all except the lowermost branchlets of the panicles for the former clone were brown and withered. The other five clones similarly treated showed more severe if not complete inhibition of normal elongation and/or floral development. GALL stated that there is a definite relationship between photoperiod and normal development and elongation of culms and panicle initials. The number of "about six" elongated internodes reported by GALL is lower than the mean number observed for any one of the ten

clones during this investigation. Those few fertile shoots tagged in 1949 and 1950 which had an elongated internode number low than that of the means of their respective clones tended, in general, to be only weakly reproductive, as shown by spindly culms and sparsely developed panicles. Despite incomplete evidence, it is possible that a relationship may exist between low numbers of elongated internodes and poor reproductive development; this needs further investigation.

Actually the number of elongated internodes per tiller may not in itself be important except in its relation with the number of living leaves present on the shoot during the year of elongation and flowering; internodal elongation does not occur between nodes at which basal leaves are produced during the previous year. The leaves expanding during the year of flowering presumably are of primary importance in the reproductive response ultimately observed. Possibly the effect of leaf number may be related to food synthesis required for normal development of the panicle or through photoperiodic "hormonal" effects. It is not clear, however, whether the shoots with fewer elongated internodes than the means of their clones are prevented from full reproductive activity because of the small leaf number, or whether the small number of leaves and the incomplete reproductive response are independent expressions of some inner physiological state.

Although the exact interrelationship of stem elongation and floral initiation in smooth brome grass is not obvious, it seems that, when the photoperiod becomes sufficiently long to allow internodal elongation, the growing points of vegetative shoots still present do not thereafter become reproductive in spite of the large number of leaves which eventually are produced. However, the long photoperiod then does promote optimal development of floral primordia already present (**9**). If the required number of green leaves is not present before internodal elongation occurs, then the shoot remains vegetative even though at a later time the leaf number becomes greater. There is, however, no particular number always found before a tiller becomes reproductive; rather these numbers fell within a range (5–14) which indicated that under different conditions and in different clones the minimal number will vary. Internodal elongation occurred only between the leaves green at some time during the year of flowering on all shoots which were dormant over the winter period. The extent to which leaves green in 1950 on shoots emerging in 1949 were initiated during 1949 or 1950 was not determined. Probably for these shoots many of the leaves green in 1950 were actually initiated during 1949.

Many shoots which emerged during the early spring of 1950 undoubtedly represented tillers arrested in development by winter cold while still below the ground surface. On these shoots some leaf primordia initiated during 1949 were undoubtedly present below ground and subjected to winter chilling. Some of these shoots were able to attain the "ripeness-to-flower" state as shown by their flowering during 1950. Most shoots which both emerged and became reproductive during 1950 were produced from basal axillary buds on culms also flowering in 1950; however, it was not conclusively shown that shoots arising from rhizome buds in early 1950 did not become reproductive. It is possible that the stimulus to flower may have been supplied by the parent tillers to those tillers emerging in 1950 which became fertile (weakly so in many cases); the parent

tillers also flowered in 1950 after emerging in the summer or autumn of 1949. For many clones most tagged tillers emerging during early spring of 1950 failed to survive (table 4). This, along with the observed lack of vigor for many fertile and sterile shoots emerging in spring, 1950, indicated that shoots which emerge in the spring do not contribute so significantly to the total growth and development of the clones as do shoots which emerge in the preceding summer or autumn; these latter shoots also appear in greater number.

Summary

1. Clones of diverse origin and type of smooth brome grass (*Bromus inermis* Leyss.) were studied in the experimental plots of the Department of Botany, University of Chicago, from October, 1949, through July, 1950, and from July, 1951, through March, 1952, to determine relationships between reproductive activity and phases of development in individual tillers, especially relative to differences in the ecological cycle for tillers initiated and growing at different seasons.

2. For ten clones studied intensively, approximately 1300 tillers were tagged in autumn, 1949, and spring, 1950, so as to provide maximum variation in size and condition. Data were recorded twice in autumn, 1949, for tillers emerged and tagged in 1949 and at harvest, July, 1950, for tillers emerged and tagged in both fall, 1949, and spring, 1950. Other tillers were dissected in spring, 1950, to determine the initial dates of floral initiation and internodal elongation. At harvest in July, 1950, the tagged shoots were grouped into four categories according to status—whether fertile, sterile, dead, or injured. Means, ranges, and frequency of distribution within the ranges were computed for data recorded for each category at every observation, according to year of tiller emergence.

3. Data obtained for individual tillers varied according to time and method of observation—whether fall, spring, or summer, and counting without dissection, or counting with dissection. The following data were recorded for individual tillers at one or more of the observational periods: position within clone; number of elongated internodes, if any; total number of leaves; distribution of leaves into classes—expanded dead leaves, visible green leaves, and green leaves, including primordia, still inclosed within the sheaths; and stem height. An evaluation of relative favorableness of growth conditions was made for each tiller tagged in fall, 1949, at the first observation; this was correlated with its condition at harvest. Thus, life-histories of tillers initiated and growing at different seasons and under different conditions were determined.

4. The clones ranged from highly fertile to relatively sterile as based on percentages of tillers producing inflorescences. Such relative fertility or sterility is in part genetically determined; however, the positions of individual tillers within the clump and the associated favorableness of growth conditions are apparently of importance in reproductivity for those clones which tend to be sterile. Centers of clumps were less favorable for growth and reproductivity than peripheral regions as shown by the number of tillers tagged in fall, 1949, which were dead or vegetative at harvest.

5. For individual tillers, fertile or sterile at harvest, considerable variation within and among the clones was observed for all data recorded. For shoots tagged in fall, 1949, the mean number of leaves exserted during 1949 was greater for tillers fertile at harvest (7.8) than for

those that were sterile (6.8); the same was generally true for individual clones. Much variability in actual number of exserted leaves present in fall, 1949, was noted for individual shoots: 2–15 and 3–12 for those fertile and sterile at harvest, respectively.

6. A considerable variation in autumnal leaf number of tillers allowed reproduction within either highly fertile or relatively sterile clones. For highly fertile clones the tendency to flower was so pronounced that individual shoots with small numbers of leaves exserted in the autumn became reproductive eventually even under unfavorable growth conditions. Within relatively sterile clones the shoots with greater numbers of leaves exserted in the autumn more often initiated floral primordia than those with fewer numbers. Nevertheless, tillers in the relatively sterile clones with small numbers of leaves exserted in fall, 1949, did, in some cases, reach a state of development necessary for floral initiation in the spring.

7. Data obtained by preharvest dissection of shoots emerging during both 1949 and 1950 indicated that a minimal variable number (about 5–14) of green leaves (including primordia) is present on a tiller during the year of actual flowering before floral initiation occurs. For approximately 80% of all fertile culms this number ranged from 7 to 10. Data also indicated that a minimal number (also variable) of green leaves must be present on an individual tiller before internodal elongation begins, or else the tiller remains vegetative, even though at a later time the leaf number becomes greater.

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LITERATURE CITED

1. ALLARD, H. A., and EVANS, M. W. Growth and flowering of some tame and wild grasses in response to different photoperiods. Jour. Agr. Res. **62**:193–228. 1941.
2. BENEDICT, H. M. The inhibitory effect of dead roots on the growth of brome grass. Jour. Amer. Soc. Agron. **33**:1108–1109. 1941.
3. BROWN, E. M. Smooth brome grass in Missouri. Missouri Agr. Exp. Sta. Circ. 243. 1942.
4. COOK, C. W. A study of the roots of *Bromus inermis* in relation to drought resistance. Ecology **24**:169–182. 1943.
5. EVANS, M., and WILSIE, C. P. Flowering of brome grass, *Bromus inermis*, in the greenhouse as influenced by length of day, temperature, and level of fertility. Jour. Amer. Soc. Agron. **38**: 923–932. 1946.
6. EVANS, M. W., and GROVER, F. O. Developmental morphology of the growing point of the shoot and inflorescence in grasses. Jour. Agr. Res. **61**:481–520. 1940.
7. FROLIK, A. L., and NEWELL, L. C. Brome grass production in Nebraska. Nebraska Agr. Exp. Sta. Circ. 68. 1941.
8. FUELLEMAN, R. F., and BURLISON, W. L. Pasture studies of brome grass, *Bromus inermis* Leyss. Jour. Amer. Soc. Agron. **33**:883–892. 1941.
9. GALL, H. J. F. Flowering of smooth brome grass under certain environmental conditions. BOT. GAZ. **109**:59–71. 1947.
10. GARDNER, F. P., and LOOMIS, W. E. Thermal induction in grasses. Abstract of paper presented at meeting of Amer. Soc. Plant Physiol., Minneapolis, Minn. Sept. 12, 1951.
11. KEYSER, A. Variation studies in brome grass (a preliminary report). Colorado Agr. Exp. Sta. Bull. 190. 1913.
12. KNOBLOCH, I. W. Morphological variation and cytology of *Bromus inermis*. Bull. Torr. Bot. Club **70**:467–472. 1943.
13. METCALFE, D. S. Physiologic responses of *Bromus inermis*. Unpublished Ph.D. thesis. Iowa State College Library. 1950.

14. MYERS, H. E., and ANDERSON, K. L. Bromegrass toxicity vs. nitrogen starvation. Jour. Amer. Soc. Agron. **34**:770–773. 1942.
15. NEWELL, L. C., and KEIM, F. D. Field performance of bromegrass strains from different regional seed sources. Jour. Amer. Soc. Agron. **35**:420–434. 1943.
16. NUTMAN, P. S. Studies in vernalisation of cereals. VII. A study of the conditions of formation and the subsequent growth of drawf embryos of rye. Ann. Bot. (N.S.) **5**:353–374. 1941.
17. OLMSTED, C. E. Growth and development in range grasses. V. Photoperiodic responses of clonal divisions of three latitudinal strains of side-oats grama. BOT. GAZ. **106**:382–401. 1945.
18. ———. Unpublished data.
19. PIPER, C. V. Cultivated grasses of secondary importance. U.S.D.A. Farmers' Bull. 1433. 1934.
20. PURVIS, O. N. An analysis of the influence of temperature during germination on the subsequent development of certain winter cereals and its relation to the effect of length of day. Ann. Bot. **48**:919–955. 1934.
21. PURVIS, O. N., and GREGORY, F. G. Studies in vernalisation of cereals. I. A comparative study of vernalisation of winter rye by low temperature and by short days. Ann. Bot. (N.S.) **1**:569–591. 1937.
22. RICE, E. L. Growth and floral development of five species of range grasses in central Oklahoma. BOT. GAZ. **111**:361–377. 1950.
23. SHARMAN, B. C. Onset of reproductive phase in grasses and cereals. Nature **150**:208–210. 1942.
24. ———. The biology and developmental morphology of the shoot apex in the Gramineae. New Phytol. **46**:20–34. 1947.
25. SPRAGUE, V. G. The relation of supplementary light and soil fertility to heading in the greenhouse of several perennial forage grasses. Jour. Amer. Soc. Agron. **40**:144–154. 1948.
26. TSIANG, Y. S. Variation and inheritance of certain characters of brome grass, *Bromus inermis* Leyss. Jour. Amer. Soc. Agron. **36**:508–522. 1944.
27. WALDRON, L. R. Some physical and chemical studies of certain clones and sibs of brome grass. North Dakota Agr. Exp. Sta. Bull. 152. 1921.
28. WATKINS, J. M. The growth habits and chemical composition of brome grass, *Bromus inermis* Leyss., as affected by different environmental conditions. Jour. Amer. Soc. Agron. **32**:527–538. 1940.
29. WHYTE, R. O. Crop Production and Environment. Faber & Faber, London. 1946.
30. ZHEREBINA, Z. N. Essay of a botanical-agronomical study of awnless brome grass (*Bromus inermis* Leyss). Trudy Po Prikl. Bot., Genet. i Selek. (Bull. Appl. Bot., Genet., and Plant Breeding) **25**:201–352. 1931.

THE RELATION OF SOIL MOISTURE TO CLEISTOGAMY IN STIPA LEUCOTRICHA

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Introduction

Among facultatively cleistogamous species many environmental factors may limit or enhance the cleistogamic condition; when so controlled, it has been called "ecological cleistogamy" by UPHOF (7). Most studies of such effects have been little more than observations; few critical experiments have been reported in which a controlled range in values of a single environmental factor has been correlated with quantitative variation of the cleistogamy ([8], for light; [1], for mineral salts). Nevertheless, the observed differences between cleistogamous and chasmogamous flowers in many reported cases of ecological cleistogamy are considerable and are outstanding examples of environmental modification of hereditary traits. In such cases the cleistogamic flowers develop no further than the bud stage with great modification of petals and reproductive organs. For example, among grasses it has been found that anthers, pollen sacs, stamens, stigmas, lodicules, and pollen diameter may be greatly reduced. CHASE (3) stated that axillary cleistogamous flowers of some grasses were so different from the chasmogamous ones that they would

not have been classified in the same tribe of the grass family. Associated with the failure of the bud to open is some method of autogamy as well as modification of the time of development of floral parts.

BROWN (2) suggested in an earlier study of cleistogamy in *Stipa leucotricha* Trin. and Rupr. that in this species this condition might be the result of low available soil water. The present study was designed to test this observation.

Material and methods

The plants of *S. leucotricha* used were derived from a single clump. This origin does not mean that all fragments of the clump are parts of a single clone, for this species typically produces axillary cleistogenes in basal leaf sheaths (2, 4) which often germinate *in situ*, producing a dense clump of numerous individuals, each of which further increases by tillering.

For planting, both 9-inch unglazed clay pots and 9-inch glazed crocks were used, each type having a drain hole in the bottom. Each container held four plants and $17\frac{1}{2}$ pounds of black clay topsoil from a small area of the cultivated experimental field of the Plant Research Institute of the University of Texas. This soil is derived from the underlying Austin chalk. The field capacity of this soil was determined by soaking an area of the cultivated field with a rotating lawn sprinkler for 12 hours, taking a sample the next morning, weighing it, drying it in an oven to constant weight at 105° C., and reweighing it. Moisture content was calculated as a percentage of dry weight. The permanent-wilting percentage was determined by growing seedlings of tomato, sunflower, and oats in sealed pint ice-cream containers until permanent wilting of the plants was reached, after which the soil was weighed, dried, and again weighed. Field capacity was 33.0%, and the permanent-wilting percentage was 13.5%.

The experimental setup required that different containers have soil containing definite amounts of available water. Since it seems impossible to maintain a given sample of soil at a constant percentage of field capacity (6), the method used to control the amount of soil water was to let the weight of the soil-filled pot or crock decrease by evaporational and transpirational loss of water to a predetermined point. The soil-water content was then raised to field capacity and again allowed to dry down to the predetermined weight. The porous pots lost water very rapidly, but the glazed crocks held soil water for much longer periods. For these reasons both pots and crocks were used.

To provide different soil-water conditions, eight pots and eight crocks were used to make two sets of four each. One set of four pots and four crocks was set out in the open sunlight; the other set was placed beside a wall with a south exposure. There was some difference in total light received, but it is not likely that light was a critical limiting factor for either set of plants. A removable waterproof cover was placed over each set of plants only when it rained or when rain was expected. With this procedure occasional rains did not interfere with the drying-out plan.

Soil treatment was varied for each container in a given set of four pots or crocks: (*a*) soil moisture was maintained near field capacity; (*b*) the soil was allowed to dry down to a mid-point between field capacity and the wilting percentage; (*c*) the soil was watered when plants reached permanent wilting; and (*d*) the soil was periodically dried down to

well below the wilting percentage, nearly to air dryness.

Plants were potted in October and kept well watered until January 1, when the various watering procedures were begun. Inflorescences were collected on April 27, when anthesis was at its peak, and each floret was examined. A diagram of each inflorescence depicting the relative position of each floret was drawn, and, as each floret was examined, the proper floret on the diagram was left open to represent the chasmogamous condition or blackened to represent the floret as cleistogamous (fig. 3*A*, *B*). Only one floret in the 545 studied appeared to be intermediate; all others were either cleistogamous or chasmogamous.

Observations and discussion

It is probably impossible to describe *S. leucotricha* as either cleistogamous or chasmogamous; it is potentially either, and both types of flowers often occur side by side in the same inflorescence. Then the differences in development of the two types are striking.

At anthesis the chasmogamous flower (fig. 3*C*) has three large anthers 3–4 mm. long, two lodicules that are swollen and 1 mm. long, and well-developed feathery stigmas about 1.5 mm. long. The ovary is very small. The lemma, its attached awn, and the glumes of both types of florets are the same. At anthesis the stamens and stigmas are forced out through the longitudinal slit of the lemma. Anthesis occurs in a large number of florets of the inflorescence at the same time, but, in general, the apical florets are further developed than the basal ones and may have been initiated earlier. At anthesis the inflorescence is well exserted from the upper sheath.

In contrast to a chasmogamous floret at anthesis is a cleistogamous floret of the same age (fig. 3*D*). The cleistogamous floret contains a grain of nearly mature size filling the cavity of the rolled lemma and with the endosperm in the "milk" stage. At the apical end of the grain is a small tangle representing the poorly developed stigmas with one to three very small detached anthers up to 0.6 mm. long. This precocity of cleistogamous flowers has been reported in *Bromus* (**5**). Autogamous pollination (**2**) occurs very early, while the inflorescence is still contained in the sheath. Evidently pollen and stigmas mature early in cleistogamous florets.

Under the conditions of this experiment, when both types of florets occurred in the same inflorescence, the cleistogamous florets were terminal, the chasmogamous florets basal, except in crock 6. This case will be described later and is unusual because of modified treatment.

Pots 1–4 were in the open, with pot 1 kept at about field capacity, pot 2 dried down to the dry weight of soil plus 23% water, pot 3 dried down to the permanent wilting percentage, and pot 4 dried down to the dry weight of soil plus 8% water. Table 1*A* lists the number and percentage of each type of spikelet produced in each of these four pots. The plants in pot 1 had produced no inflorescences at the time of collection. In general, soil kept at field capacity was too wet for best growth of this species. Figure 1*A* is a graph of the results listed in table 1. A direct relationship between chasmogamy and a high level of soil moisture and between cleistogamy and a low level of soil moisture is indicated. Table 1*B* and figure 1*B* show the results obtained from pots 5-8 that were kept against the wall with exposure to the south. It is obvious that the method of control results in soil water being variable from time to time. The time of watering relative to the time

when floral initiation took place produced some variation in the relative amounts of cleistogamy and chasmogamy (fig. 1*A*, *B*, *C*).

Crocks 1–4 yielded results (table 1*C* and fig. 1*C*) rather similar to those of the pot treatments, except that crocks 2 and 3 had more chasmogamy than pots 2 and 3. This probably results from the fact that glazed crocks hold soil moisture much longer than porous pots, and over such a period of time as 4 months the plants actually have more available water. Nevertheless, the data from plants grown in crocks also show a correlation between available soil water and the extent of cleistogamy. This correlation is also illustrated by the combined data (fig. 2) from all plants reported in table 1*A*, *B*, and *C*.

Crocks 5 and 8 were given a modified treatment. From January 1 to March 12 crock 5 received the least water and crock 8 the most. From March 12 to April 27 crock 5 received the most water and crock 8 the least. Although no observations were made, it is fairly certain that flower primordia were formed shortly after March 12. The plant in crock 5 received more than sufficient water up to March 12, but, since drying out of soil in glazed crocks is slow, there was ample moisture at the time of floral initiation to result in all chasmogamous florets. The plants in crock 8 received little water prior to March 12. Subsequently,

TABLE 1

NUMBER AND PERCENTAGE OF CLEISTOGAMOUS OR CHASMOGAMOUS FLORETS FROM EACH CONTAINER

	A				B				C				D			
	Pot 1	Pot 2	Pot 3	Pot 4	Pot 5	Pot 6	Pot 7	Pot 8	Crock 1	Crock 2	Crock 3	Crock 4	Crock 5	Crock 6	Crock 7	Crock 8
No. cleistogamous	....	18	14	15	8	34	33	20	.....	.....	26	15	.....	23	19	.....
% cleistogamous	....	46	78	100	16	64	46	83	.....	.....	24	100	.....	54	37	.....
No. chasmogamous	....	21	4	....	42	19	39	4	17	20	81	.....	85	18	32	38
% chasmogamous	....	54	22	....	84	36	54	17	100	100	76	.....	100	46	63	100

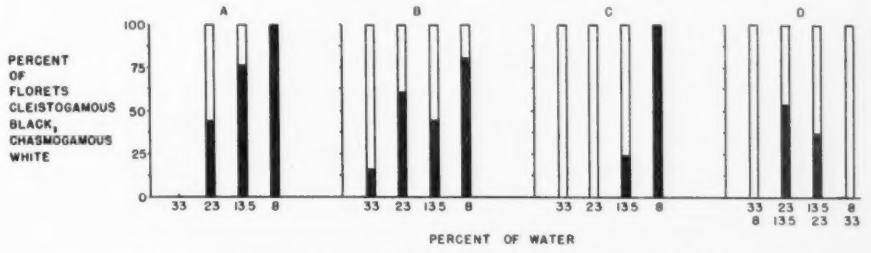

FIG. 1.—Graphic representation of data in table 1; groups of table 1 correspond with groups of fig. 1

at the time of floral initiation they apparently received ample water, for all flowers were chasmogamous (table 1*D*; fig. 1*D*). Crocks 6 and 7, however, had intermediate amounts of water both before and after March 12, and so there was at most a possible slight shift in the extent of cleistogamy of these two relative to each other. This change in treatment evidently occurred at a critical point of floral initiation in the plants in crock 6 so that the terminal florets were chasmogamous, the basal were cleistogamous. This arrangement was found in no other plants; in all others, if both types occurred in one inflorescence, the cleistogamous florets were terminal (fig. 3). This unusual arrangement may be a result of the treatment; the plants had sufficient water up to March 12 to produce chasmogamous florets but rather less thereafter and produced cleistogamous florets. According to this interpretation, the terminal florets were produced first under conditions of an adequate water supply and so were chasmogamous; but, when the lower florets were initiated, there was less soil water, and the florets produced were cleistogamous. Since the terminal florets reach anthesis a few days before the basal florets, it may well be that the terminal florets are initiated earliest. A somewhat similar condition was reported (5) in *Bromus carinatus* Hook. and Arn.

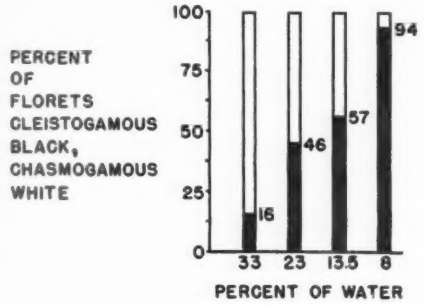

FIG. 2.—Graphic representation of combined data in table 1*A*, *B*, and *C*.

In all series of this experiment cleistogamy was inversely related to availability of soil water. It seems that this environmental factor is able to modify the action of genes determining the morphological characteristics and the time of development of the floral parts. It is evident that there is a fine balance between the cleistogamous and chasmogamous tendencies, such that a change in the quantity of soil water can change the character of florets in the middle of an inflorescence. It is not known why, in in nearly all plants with both types of florets, the cleistogamous ones are terminal, but it seems likely that the time of watering about the period of floral initiation may be of significance. It is also unknown why, in all cases except one, each floret is completely cleistogamous or chasmogamous. The only floret that appeared intermediate was found on an inflorescence from the plant in crock 6, the inflorescences of which were unusual in other ways.

HARLAN (5) postulated for *Bromus carinatus* that it is probable that the chasmogamy or cleistogamy of a floret is determined at the time of differentiation in the primordium with an abrupt physiological transition possible that might be explained on a threshold basis. The evidence presented here suggests that this concept might apply also to *Stipa leucotricha* in relation to available soil water alone.

Summary

1. Plants of *Stipa leucotricha* were grown under four different conditions of available soil water in porous unglazed clay pots and in glazed crocks. In all cases the less the amount of soil water

available to a plant, the higher its percentage of cleistogamy.

2. This species is evidently neither chasmogamous nor cleistogamous but is potentially either. The balance between these two conditions is delicate and can be changed during floral initiation by the amount of available soil water crossing a determining threshold value.

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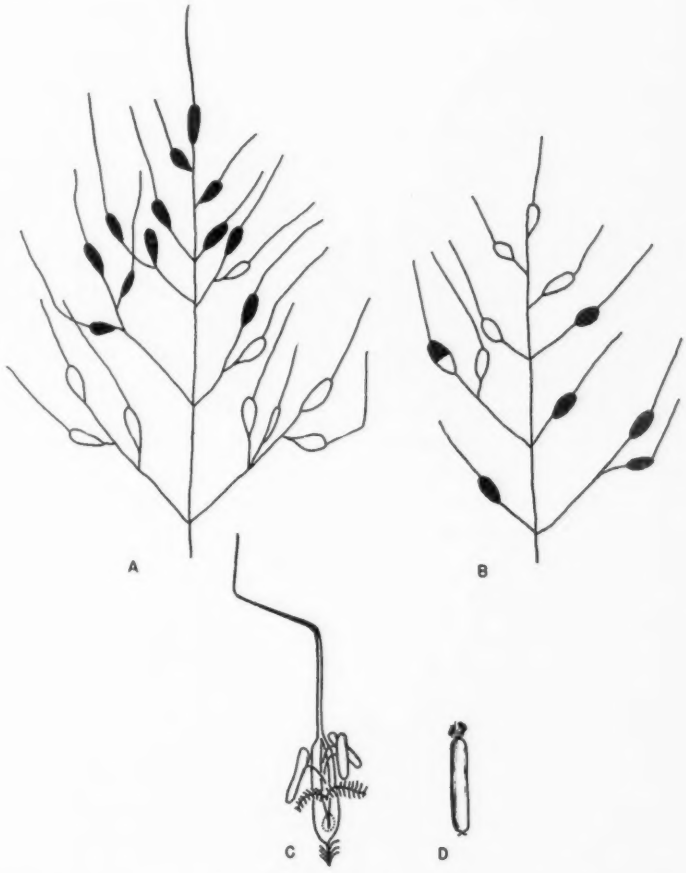


FIG. 3.—*A*, diagram of typical inflorescence consisting of both cleistogamous (black) and chasmogamous (white) spikelets, the cleistogamous terminal. From pot 6. *B*, diagram of inflorescence from crock 6, with cleistogamous florets basal. One floret was intermediate as to anther and stigma and was sterile. *C*, chasmogamous floret at anthesis. *D*, cleistogamous grain from floret of same age as *C*. Note two small anthers tangled in small stigmas in cleistogamous floret.

LITERATURE CITED

1. BERGDOLT, E. Morphologische und physiologische Untersuchungen über *Viola*, zugleich zur Lösung des Problems der Kleistogamie. Bot. Abh. **20**:1–120. 1932.
2. BROWN, W. V. A cytological study of cleistogamous *Stipa leucotricha*. Madroño **10**:97–107. 1949.
3. CHASE, A. Axillary cleistogenes in some American grasses. Amer. Jour. Bot. **5**:254–258. 1918.
4. DYKSTERHUIS, E. J. Axillary cleistogenes in *Stipa leucotricha* and their role in nature. Ecology **26**:195–199. 1945.
5. HARLAN, J. R. Cleistogamy and chasmogamy in *Bromus carinatus* Hook. and Arn. Amer. Jour. Bot. **32**:66–72. 1945.
6. KRAMER, P. J. Plant and Soil Water Relationships. McGraw-Hill Book Co., New York. 1949.
7. UPHOF, J. C. TH. Cleistogamic flowers. Bot. Rev. **4**:21–49. 1938.
8. VOCHTING, H. Über den Einfluss des Lichtes auf die Gestaltung und Anlage der Blüte. Jahrb. wiss. Bot. **25**:186–187. 1893.

VEGETATIVE ANATOMY OF THEOBROMA CACAO

ERWIN R. BROOKS AND ARTHUR T. GUARD

Introduction

Cacao, *Theobroma cacao* L., is a small evergreen tree, native to the American tropics. According to BARRETT (1) 98% of the world's chocolate and cocoa supply is made from its seeds.

Since this species plays an important role in the economy of the world, it seemed desirable to undertake a careful study of its morphology and anatomy. Such information would be useful as a background for practices used in its culture, as well as further knowledge of the structure of a member of the Sterculiaceae.

All the material used in this anatomical study of cacao came from the Inter-American Institute of Agricultural Science at Turrialba, Costa Rica, or its cacao experimental plantation, La Lola, at Madre de Dios, Costa Rica.

Observations

ROOT

T. cacao has a prominent taproot. A 15-foot tree dug and measured at the La Lola Plantation had a taproot more than 5 feet long. Soil where this tree was growing was a very heavy, stony, lateritic clay containing many large boulders. Most of the large feeder roots are usually within the top 4–6 inches of the soil surface. Most of the lateral roots arise at the soil surface from a definite ring zone on the primary root. This prominent feature appears at the transition zone in the germinating seed.

The surface laterals form a dense mat for several feet around the trunk. For the tree cited above, one lateral extended 16 feet. It can be readily seen, therefore, that orchards planted with trees at the usual distance of 12 feet apart have a continuous mat of interlacing feeder roots in the surface soil. This should be kept in mind in cultivating and in fertilizer experiments.

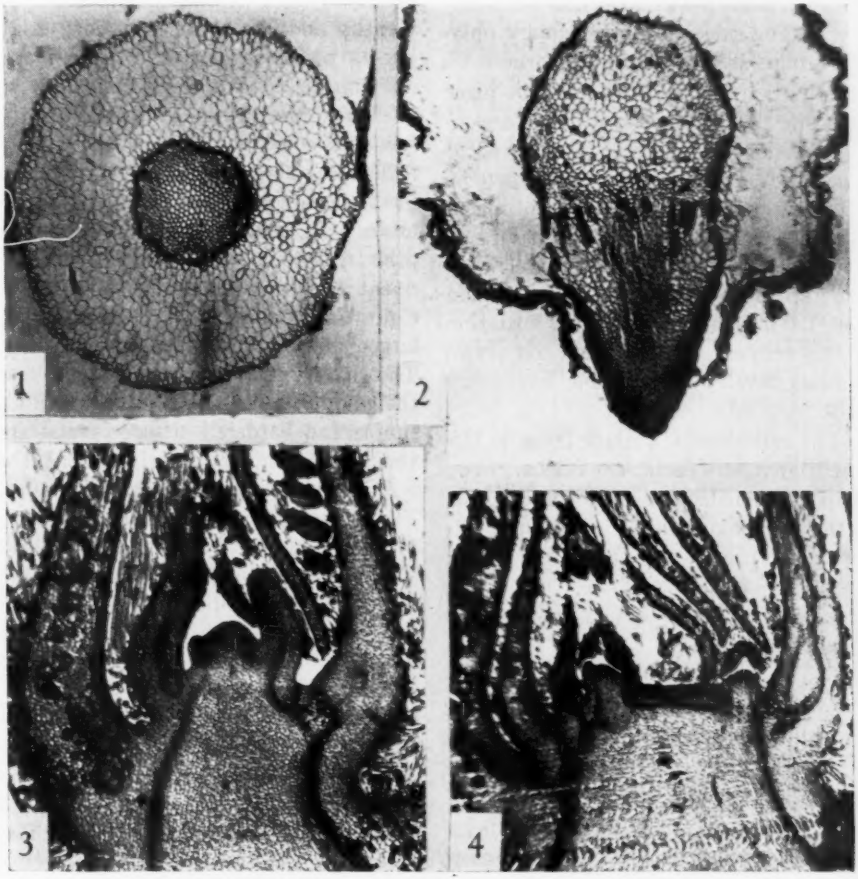
PRIMARY TISSUES.—The primary root is hexarch (fig. 1). The metaxylem strands widen as they approach the center, but union of the strands is not achieved. No definite annular or spiral elements were found in the protoxylem.

Very prominent in the root tip are certain cells which are filled with a mucilaginous material and stain heavily. They elongate considerably more than do the surrounding cells; and the end walls disappear. They extend up into the cen-

tral parenchyma and metaxylem as solitary strands which are straight, unbranched, and decrease in number above $\frac{1}{2}$ cm. from the tip until there are few or none above 1–2 cm.

At from 1 to 5 cm. from the root tip the parenchyma cells in the center and between the metaxylem strands are only slightly elongated. These thin-walled cells have large vacuoles, and occasional ones are filled with mucilage.

On alternate radii to the primary xylem strands are six groups of primary phloem which are separated from the more centralized metaxylem by parenchyma. These primary phloem groups are separated from the endodermis by at least a single layer of pericyclic cells. As the primary phloem cells are parenchymatous and only slightly smaller than the parenchyma cells, they are quite hard to distinguish. As maturation of the tap-



FIGS. 1–4.—*Theobroma cacao*. Fig. 1, transverse section of primary root of 6-week-old seedling taken 3 inches from tip. Fig. 2, transverse section of primary root showing young lateral. Note that entire side of stele is involved. Fig. 3, median longitudinal section of chupon-wood primordium. Fig. 4, median longitudinal section of very young orqueta.

root proceeds, the primary phloem is eventually crushed and disappears.

The pericycle is a single layer of uniform cells around the stele, forming a hexagon in cross section. The six flat sides and six oblique corners are very striking. When the roots are grown in water culture, the sides are very regular. In soil, however, this configuration is sometimes greatly distorted. In all cases, nevertheless, the sides are straight, and the protoxylem point lies at the center of its corresponding side, while the primary phloem groups are located at the corners.

Initiation of lateral roots takes place opposite the protoxylem points. Tangential divisions of the pericyclic cells occur along the entire side. There appears to be no special sequence of lateral root initiation, although in the region of major surface laterals all six sides of the stele may show secondary-root initiation in a region 100–150 μ long. In this region initiation occurs very early in ontogeny, and, if two laterals develop at the same level, they arise from adjacent sides.

The endodermis, a single layer, is differentiated very early. The cells are filled with a mucilaginous substance that stains so heavily that Casparian thickenings, if present, are obscured. There are a few clear cells which may be passage cells, although they are not located at any regular loci.

Ten to sixteen layers of large, parenchymatous cells outside the endodermis make up the cortex. Most of the cells are clear, thin walled, and slightly elongated parallel to the root axis. An occasional cell is filled with a substance which stains heavily, and the two or three layers next to the epidermis sometimes stain so heavily as to make the individual cells indistinct.

The epidermis consists of a single layer of cells and is differentiated very early. The cells become filled with mucilage as soon as they are differentiated and continue to stain heavily until shortly before secondary tissue is formed. Root hairs do not seem to develop on the primary root until after cambial activity is evident.

SECONDARY TISSUE IN THE PRIMARY ROOT.—As the primary tissues mature, the procambium becomes more difficult to identify. This is not surprising, as the cambium itself is almost impossible to identify and can be located most easily by its position relative to xylem and phloem. In appearance it resembles an ill-defined interfascicular cambium. It is, however, continuous around the stele.

The secondary xylem consists of prominent vessels, nonlignified parenchymatous cells, and ray cells. Close to the primary xylem, vessel elements are frequent and occur in groups of three to eight. Farther out they are less frequent, larger, and occur in shorter radial rows. The thick vessel walls are lignified, and the middle lamella is prominent. Both simple and bordered pits occur and are very large and so numerous that the walls have a lattice-like appearance. This is especially true in walls common to two vessels or to a vessel and a wood parenchyma cell which lies radial to the vessel. The end walls of vessel elements are washer-like, with a single large perforation in the center, and are slanted at a slight angle. Rarely an end wall may be slanted at more than 45°.

The two types of pits may both occur in a single vessel element. The simple pits are lanceolate-ovate to oval in shape, the former being much more common. The bordered pits have greatly reduced borders, and the opening mouth is slitlike. As would be expected, they occur as pit-pairs and are most abundant in vessels and cells associated with vessels. Unex-

pectedly, pits are few and very elementary in the ray cells.

Xylem rays are numerous, one to three cells wide, ten to forty cells in depth, with single rows of marginal ray cells at upper and lower edges. The ray cells are roughly rectangular in cross section, the axis radial. The end walls are perforated with numerous, large pits. The side walls are thin and contain few pits. Starch grains are numerous, and many of the cells stain solidly as a result of the mucilaginous material they contain. Most vessels are in direct or close contact with a ray.

As would be expected, each ray continues into the phloem. Here the ray widens, making the phloem portion of the ray triangular, with the apex toward the cambium. This characteristic is common in the Sterculiaceae.

The secondary phloem consists of sieve tubes, companion cells, phloem fibers, and phloem parenchyma, in addition to the phloem rays. These all occur in a definite pattern. In later development phellogen arises in the phloem region.

The sieve tubes are small, irregular in cross section, and somewhat elongated. Most of them have a transverse end wall which contains the sieve plate. A single sieve tube may abut on one or two others. The side walls are thin, and no lattices have been found. A few sieve tubes contain starch grains, mucilage, or slime plugs. Most of them, however, appear clear and functional.

Companion cells are difficult to recognize because of the small size of all phloem elements in the root of the cacao. They are smaller than the sieve tubes and of about the same length. The end wall angles as it abuts the next companion cell. The cell is clear, contains a small nucleus, and appears to remain in a meristematic condition. The sieve tubes and their companion cells form groups, centrifugal to the cambium and centripetal to bundles of phloem fibers, and are bounded radially on both sides by the phloem rays.

The phloem fibers are much elongated, pointed, and small in diameter. The cell walls are thick and lignified, and the lumen is small. The fiber bundles frequently appear triangular in shape in transverse section as a result of the tangential expansion of the phloem rays. Small groups of isodiametric parenchyma cells and crushed sieve tubes are intermixed within the fiber bundles.

Cork cambiums may be initiated at different depths at approximately the same time. The outermost is in the cortex, and two or three more layers may be formed there before the secondary phloem is reached. The phellogens are often only one to three cells apart. The cork cells, heavily suberized and with lumens completely filled, form a tissue which is perfectly regular with no intercellular spaces. No phelloderm has been found even when phellem layers were fifteen to twenty cells in depth. The entire mature root is encased in these cork layers. The outermost layer is eventually sloughed off, and a newer one takes its place.

SECONDARY OR LATERAL ROOTS.—As stated earlier, the initiation of secondary roots takes place in the pericycle. The new root pushes out along one whole side of the pericyclic hexagon (fig. 2), breaking the endodermis, cortex, and epidermis of the primary root as it grows out to the soil.

The regions of the lateral roots close to the tip seem identical with those of the primary root. The major difference between the two is found in the primary vascular strands. Whereas the primary root is hexarch, the lateral roots are tetrarch.

Formation of secondary elements

rounds out the xylem and crushes the primary phloem. Branch lateral roots are also initiated in the pericycle outside the xylem arms.

The epidermis in lateral roots, unlike that in the primary roots, remains clear. Root-hair formation does not start until after lateral roots are produced.

Anatomy of the other tissues of the secondary root is essentially the same as in the primary root.

Shoot

The stem is dimorphic; the main trunk varies in height from 4 to 10 feet. The senior author observed one orchard in heavy shade and with thick underbrush where the trunks were 15–20 feet high. The trunk ends in a crown of five branches known as an orqueta. The internodes between these five branches are so telescoped that, after 2–3 years of growth, they appear as if they had arisen from one node. This orqueta formation is one of the most outstanding characteristics of *T. cacao*. It differs from ordinary crown formation in that it is formed naturally rather than induced by pruning; unless modified by pruning or injury, five branches are always formed, and the phyllotaxy of the stem below the orqueta is $\frac{3}{8}$ and above it is $\frac{1}{2}$. Cook (**2**), in an excellent description of the foliage characteristics, has reported the phyllotaxy of the upright shoots as $\frac{5}{12}$.

Trunks below the orqueta give rise only to chupons, which are shoots morphologically the same as the trunk. They likewise have the $\frac{3}{8}$ phyllotaxy and terminate in an orqueta. The branches of the orqueta or fan wood, as they are generally called, may give rise to more fan wood or, occasionally, to one or more chupons. The formation of chupons on the fan wood is greatly stimulated by pruning or wounding.

The chupons always make a right angle in their attachment to the trunk or branch from which they arise. At the base of this angle is a root primordium which, if the chupon is within 4 inches of the ground, will develop into a root which enters the soil and behaves like a taproot. After this has become established in the soil, the attachment of the chupon to the parent tree may break and the new plant continue as an independent tree. Vegetative reproduction by this method is common in fallen trees.

Leaves.—Dimorphism of the leaves is not so pronounced in *T. cacao* as in some other species of *Theobroma*. The petioles of the leaves of trunks or chupons are somewhat longer than those of the branches. Also the vigor and general size of the chupon leaves are usually greater than in fan-wood leaves. Cook (**2**) stated that the petioles are about 1 inch long on the lateral branches and 3–4 inches long on the upright shoots. He also showed that, although petiole length varied with leaf size, the ratio of the petiole length to blade size is almost always greater in leaves of the upright shoot.

The leaf petioles have basal and apical pulvini which tend to regulate the position of the leaves. The degree of their activity varies from tree to tree. This variation may account in part for the ability of some trees to survive better than others when not shaded.

The leaf blades are simple, lanceolate to lanceolate-ovate. The margin is entire and slightly wavy. The venation is pinnate, and the surface is glabrous on both sides.

A bud is borne in the axil of each leaf; it remains dormant until long after the leaf has dropped. Cook (**2**) stated that there appear to be adventitious inflorescences at nodes where no leaves have de-

veloped or, at least, where no leaf scar could be found. It seems to the authors that the latter is more likely the case. Two factors strengthen this view: (*a*) The authors have never found inflorescences on the fan wood which did not follow the $\frac{1}{2}$ phyllotaxy of the leaves. Trunks and chupons were not checked, however. (*b*) Incomplete leaf development toward the latter part of the growth flush causes the location of the nodes to be obscure. Since flowering cushions form later, it is difficult to ascertain the position of the node.

HUMPHRIES (**3**) and HUMPHRIES and MCKEE (**4**) have studied the cycle and causes of growth flushes in cacao. They found that at least 8 weeks had to elapse between flushes in the same twig. Not all trees within an area will grow at the same time unless a definite dry-and-wet seasonal cycle prevails. They also found a correlation between flushes and maximum air temperature, provided an 8-week period had elapsed. There are usually two to four heavy flushing periods a year per tree.

FLOWERING CUSHIONS.—The heaviest flowering and fruiting always take place on the oldest wood therefore, on the trunk. Flowering may occur on wood that is only three or four flushes old, and occasionally small fruits develop from these flowers.

The flowers occur on extremely telescoped fruiting branches called flowering cushions. As the flowering cushion becomes older, it widens until it looks like a knot or hump about 4–5 inches wide and raised 1–2 inches from the trunk or main branch. Such a flowering cushion may have from forty to sixty flowers on it at one time and be able to mature four large fruits during a single fruiting period, though such cases are rare. Generally a tree produces several thousand flowers annually but matures only a very small percentage of fruit.

ANATOMY OF STEM.—Although the stem is definitely dimorphic, the anatomy of both chupon and fan wood is fundamentally the same. Very young stems of both are terete. The $\frac{3}{8}$ phyllotaxy of the chupon wood is indicated by the irregularity of the occurrence of leaf primordia in median longitudinal section (fig. 3).

The provascular tissue of both types of stems forms a continuous ring, and maturation of the protoxylem occurs very close to the apex.

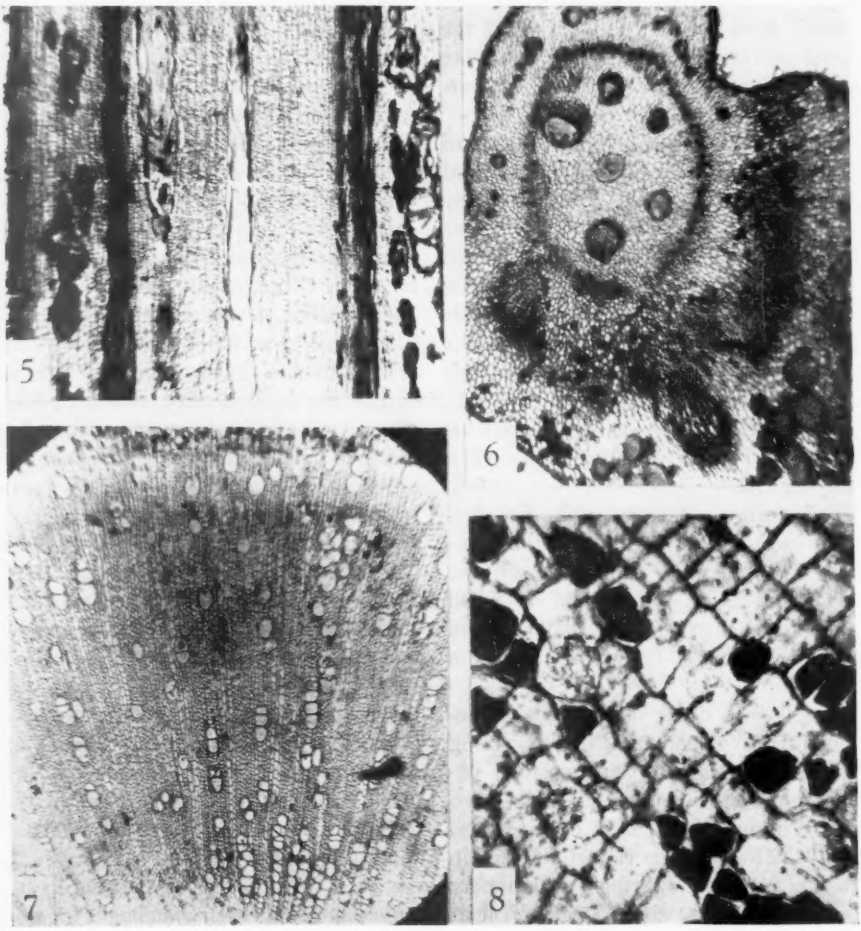
At the nodes maturation of vascular elements begins in the leaf trace and proceeds both basipetally and acropetally. The primary xylem is endarch and usually shows a radial alignment continuous with that of the secondary xylem. The phloem components are so small that it is hard to distinguish the characteristics of different cells. Most of them are parenchymatous and isodiametric, although a few are slightly elongated. Some contain either starch grains or mucilaginous substances.

The pith is rather large, circular, and persistent. The cells are large and isodiametric with intercellular spaces. Of unusual interest are the large, elongated, lysigenous cavities. The position of these cavities is indicated close to the stem tip by groups of cells in which the cytoplasm is more granular and has a greater affinity for stain. At this stage these cell groups form a pattern of one central group surrounded by five other equally spaced ones. Each of these groups rapidly develops into a large lysigenous cavity which is filled with mucilage (fig. 6). Each extends for a considerable distance and perhaps through the length of the stem (fig. 5).

The pericycle and endodermis are ex-

tremely hard to distinguish. Many of the innermost cortical cells, more elongated longitudinally than the other cells of the cortex, fill with mucilage; there is no definite proof that they are endodermal cells, however.

The cortex is usually from fifteen to twenty layers of cells in thickness. All the cells are essentially parenchymatous in nature with the cells of inside layers isodiametric and those toward the outside somewhat elongated and smaller. Cells in the two or three layers centripetal to the epidermis are filled very early in their development with a mucilage-like substance. No collenchyma cells are formed in either fan or chupon wood except at the base of the leaf petioles.



FIGS. 5–8.—*Theoboma cacao*. Fig. 5, radial section of young fan wood showing lysigenous cavities of pith and cortex. Fig. 6, transverse section of fan wood taken very close to stem apex. Note lysigenous cavities in pith and cortex and stipule and petiole vascular traces. Fig. 7, secondary xylem of stem in transverse section. Fig. 8, brick-shaped cortical cells in pulvinus. Small dark spots are starch granules.

Many mucilage-filled, lysigenous cavities are also found in the cortex and are continuous into the leaf petioles and stipules. The strands are apparently not so long as in the pith and are generally smaller, more numerous, and more irregular. The irregularity probably results from two or more cavities uniting

The epidermis is the first tissue differentiated. The cells are small, uniform, and clear, and the walls a little thicker than those of the cortical cells. Unicellular, pointed, epidermal hairs occur profusely, especially on the terminal portions of the stem and on stipules, petioles, and buds. The type of epidermal hair is very characteristic of the species and is used in taxonomic identification. These hairs are formed by the prolongation of the outer wall of the epidermal mother cell. Many are quite long and are often filled with starch grains or a mucilaginous substance. A few of the hairs and the epidermis persist for a considerable time after initiation of secondary growth. Only after considerable phellem is produced is the epidermis finally sloughed off.

ORQUETA FORMATION.—Anatomical examination of the orqueta shows that each branch arises from an individual node, but these nodes are so telescoped as to appear almost as a single one (fig. 4).

A marked lack of internodal elongation is the first sign that an orqueta is forming. The branches develop rapidly from the axillary buds, and the subtending undeveloped leaves and stipules abscise. Before the branches are an inch long, only vascular traces are left to show that leaves and stipules ever existed.

The question arises as to whether axillary buds on the chupon wood below the orqueta would produce fan wood, like the branches at the orqueta, or chupon wood. In each leaf axil only one bud develops, whether that axil is on chupon or fan wood, and all appear identical, with a phyllotaxy of $\frac{1}{2}$.

If one of the five lateral branch buds was not allowed to develop, would a lower bud take over as a fan-wood branch? This cannot be answered until more work is done. If, as anatomical examination would indicate, buds below the orqueta are fan buds, the origin of chupons becomes the next question. The supposition that they arise from adventitious buds appears logical but needs proof. Evidences to support an adventitious origin are the occurrence of chupons on fan wood and their abundance after a heavy pruning.

In the chupon wood two vascular traces leave the stele and pass to the stipules. Between them and at a slightly higher level a third trace breaks from the vascular cylinder and enters the petiole (fig. 6). The vascular cylinder then closes over the gap and close above forms a trace to the bud. All these traces are unbroken and uniform in their passage through the cortex. This, however, is not the case where the stem terminates in the orqueta. Here, under the cortex of the central dome, the vascular elements are highly irregular. The broken, uneven, multidirectional strands resemble those found in gall or graft tissue.

Growth of the branches causes the top-central part of the orqueta to be pushed and pinched upward into a dome. This evident growth stress has caused discussion as to the strength or weakness inherent in a crown of this type. Longitudinal sections show no splitting, however, nor do they show any more evidence of stress weaknesses than can be found in any branch crotch of a similar angle. In the sections studied this angle is great enough so that it would seem

highly improbable that phloem pockets could be formed and thus render the crotch weak.

SECONDARY STEM TISSUE.—Secondary thickening in the stem is quite similar to that in the root, and lateral growth appears to be the same in both fan and chupon wood. Secondary vessel elements are added in direct radial alignment with those of the primary xylem (fig. 7). Vessels are much more abundant in the stem than in the root, but their characteristic radial grouping is the same as in roots. The xylem fibers have rather thin lignified walls with a few simple pits. They are elongated to about ten times their diameter. The vascular rays of the stem are similar to those of the root, except that they are wider, the simple pits more noticeable, and many of the cells in the phloem portion have solitary druses of calcium oxalate.

In the stem a broad zone of similar, small, thin-walled, meristematic cells makes it difficult to locate accurately the actual cambium.

The phloem is generally thicker in the stem than in the root. Phloem fibers occur in small groups separated tangentially by phloem parenchyma or crushed sieve tubes and companion sells and radially by the phloem rays. Sieve tubes can only be identified positively by the slime plugs. They and their companion cells occur in small groups between patches of cells which later become fibers. Sieve-tube elements and companion cells are similar to those in the root.

Phellem is much less abundant and production slower in the stem than in the root. Usually there are only one or two layers of phellem as compared with the many layers in the root. Mention should be made again of the ability of the cortex and epidermis to remain undestroyed long after considerable secondary xylem and phloem have been produced.

LEAF ANATOMY.—In early leaf ontogeny the stipules develop below and on either side of the petiole with but the narrowest connections to the petiole. In development, however, enlargement of the petiole results in its downward extension and a further separation of the stipules so that the latter appear on a level with the solitary bud. This makes it probable that stipule scars may have been mistaken for additional axillary buds.

Each vascular trace to a stipule sends off a side branch toward the main trace of the petiole while still within the main stem. The two side branches do not join the main trace but turn into the basal pulvinus. At the same time the central petiole trace divides to form three strands. The central strand then passes up through the basal pulvinus, and where this merges with the regular petiole the five traces unite to form a vascular cylinder.

In the apical pulvinus the vascular cylinder acts very erratically. Side traces are formed only to rejoin the vascular cylinder. The cylinder develops gaps, flattens out, becomes U-shaped, and performs generally in an unusual manner. As they enter the midrib, however, the vascular tissues appear shaped like a half-moon in transverse section.

Pith cells throughout both pulvini, regular petiole, and midrib are very similar to those in the main stem, but only one mucilage-filled cavity is formed. The xylem consists of parenchyma and vessels. These vessels are large, mostly reticulate, with a few scalariform and spiral elements. The phloem in this region shows little differentiation. Outside the phloem are narrow bands of fibers which are continuous through the pulvini, petiole, and midrib.

The stele of the petiole is surrounded

by multilayered cortex. In the basal pulvinus this is thickest on the abaxial side, whereas the reverse is true in the apical pulvinus. The cortex also contains mucilage-filled, lysigenous cavities. The outermost layers of the cortex are made up of collenchyma cells, many of which are filled with mucilage, solitary druses of calcium oxalate, or starch granules.

The movement of the pulvini is probably attributable to the parenchymatous cortical cells. These cells are uniform, brick shaped, evenly spaced, and contain many very small granules of starch (fig. 8). The occurrence throughout the leaf of these unusually small granules is interesting. They are found in cells of the phloem, pith, cortex, and mesophyll. Their prominence in cortical parenchyma suggests that they may have something to do with the pulvinus movement.

The epidermis is made up of small regular cells and epidermal hairs which are prominent on the stipules. The surface of both sides of the pulvini, especially the apical one, which has the larger arc of movement, is very wrinkled; otherwise the leaf surfaces are smooth.

The mesophyll of the blade shows three layers of palisade cells on the adaxial side and an equally thick layer of spongy tissue on the abaxial side. The cells of the palisade mesophyll are very small, contain many plastids, and are three times longer than their diameter. With the exception of the innermost layer, the intercellular spaces in the palisade mesophyll are small. More or less spherical, small, lysigenous cavities which are filled with mucilage are very common in this layer. These cavities frequently break through the upper epidermis and exude their mucilage.

The cells of the spongy mesophyll are small and abut end to end to form solitary strands extending from the lower epidermis to the palisade parenchyma. There is considerable intercellular space.

The upper epidermis is very different from the lower. The upper has cells which are large, clear, and relatively thin walled. It is covered with cutin and is unbroken except for the already mentioned lysigenous cavities. The lower epidermis is made up of cells which are extremely small, filled with a substance that stains heavily, and are fairly thick walled. Numerous very small stomata are irregularly scattered over the lower epidermis. Both the guard cells and the stomata are extremely small.

Persistence of the leaves depends on the sun or shade environment and on the characteristics of the individual tree. Where a tree appears normal, the leaves may remain for three or four flushes. An abscission layer is formed at the base of the basal pulvinus, and the leaf is still green when it falls.

ANATOMY OF FLOWERING CUSHION.—The very cauliflorous flowering cushion is derived from the solitary axillary bud of a leaf. The vascular tissues form a cylinder. Lysigenous cavities are much rarer than in vegetative shoots, but many of the cells of the pith, cortex, and rays are filled with a heavily staining mucilage.

The basal portion of the cushion is so twisted that considerable tissue which would ordinarily be sloughed off is trapped and held. This provides a refuge for some types of insects as well as explaining, in part, the weakness of the cushion.

COOK (**2**) stated that the branches are so rudimentary that the terminal joints with two pedicels are practically sessile. He was thus unable to determine the branching arrangement. This is partly true, but anatomical examination shows that the vascular traces are dichotomous-

ly branched. Only one of the next to the last internodes elongates. This terminal internode produces two pedicels, one of which matures much faster than the other. Thus it appears as though the less mature floret is a branch of the other. The nonelongated internode also appears as a branch.

Where the terminal internode joins the pedicels, two, opposite, small, stipule-like appendages are formed. The abscission layer is located just distal to these. This layer functions if the flower is not fertilized but not if a cherelle is formed.

Summary

1. Some of the outstanding morphological features of *Theobroma cacao* are its chupon and orqueta formation, the basal and apical pulvini in petioles, and the flowering cushion.

2. The xylem of the primary root is hexarch, while that of the lateral roots is tetrarch.

3. Mucilaginous strands occur in all root tips, and mucilage-filled cells are common throughout the root system.

4. Chupon and fan woods are anatomically similar.

5. Elongated, mucilage-filled, lysigenous cavities occur in the pith throughout the shoot system, while shorter ones are very numerous in the cortex.

6. The chupon terminates in a five-branched orqueta. Anatomical examination shows a lack of internodal elongation between the nodes of these five branches.

7. There is only one bud to a leaf axil.

8. Spherical lysigenous cavities are common in the palisade layer of the leaf blade. The upper and lower epidermis are strikingly different. Stomata occur only in the lower epidermis, and the guard cells are very small.

9. A flowering cushion is derived from a solitary axillary bud.

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LITERATURE CITED

1. Barrett, O. W. The Tropical Crops. Macmillan Co., New York, 1928.
2. Cook, O. F. Branching and flowering habits of cacao and patashte. Contrib. U.S. Nat. Herb. 17. (Part 8). 1916.
3. Humphries, E. C. Dormancy of cacao buds. (I) A consideration of the factors concerned in the breaking of the rest period. Eleventh Ann. Rept. Cacao Research, Trinidad: 28–32. 1944.
4. Humphries E. C., and McKee, R. K. Dormancy of cacao buds. (II) Carbohydrate changes during the development of a flush. Eleventh Ann. Rept. Cacao Research, Trinidad: 33–36, 1944.

TAXONOMIC RELATIONSHIPS AND BREEDING POSSIBILITIES OF SPECIES OF ABELMOSCHUS RELATED TO OKRA (A. ESCULENTUS)

B. P. PAL, H. B. SINGH, AND VISHNU SWARUP

Introduction

It is well known that some genera of Malvaceae yield important economic products. One of these genera, *Hibiscus* contains some fiber plants like kenaf (*Hibiscus cannabinus*) and roselle (*H. sabdariffa*). Another species, popularly known as *Hibiscus esculentus*, is the okra or bhendi plant, whose young fruits are used as a vegetable and whose seeds are the source of an oil. In this paper certain aspects of work on okra, or bhendi, and related species have been described.

The genus *Hibiscus*, which contains a large assemblage of plants showing considerable variation, was established by LINNAEUS in 1737. Exactly a half-century later MEDIKUS (**10**) proposed the generic name *Abelmoschus* for a group of species previously described under *Hibiscus*. Some of the later workers—for example, MASTERS (**9**)—recognized *Abelmoschus* as only a section of the genus *Hibiscus*. The species of this section were characterized by a spathaceous, circumscissile, and deciduous calyx. SCHUMANN (**13**) recognized the genus *Abelmoschus* of MEDIKUS (*Hibisci* Sect. *Manihot* et *Abelmoschus* DC. ex parte) on the basis of the deciduous nature of the calyx, the genus *Hibiscus* having a persistent calyx. HOCHREUTINER (**7**), in revising the genus *Hibiscus*, however, suggested that the genus *Abelmoschus* of MEDIKUS might well remain as a section of the genus *Hibiscus*. This view was also held by several others, especially by the authors of Indian floras. COOKE (**3**), PRAIN (**12**), DUTHIE (**4**), GAMBLE (**5**), and HAINES (**6**) recognized the characters separating the species of one group from another in their keys to the species, although they did not particularly use the various sectional names. As a result the common okra, or bhendi, which belongs to the *Abelmoschus* section, is widely known in India and elsewhere as *Hibiscus esculentus* L. In a later work HOCHREUTINER (**8**), after a critical study of the relative limitations of these sections, declared—thus changing his own previous view—that the species contained within the *Abelmoschus* group are sufficiently distinct to justify being considered as a separate genus, *Abelmoschus*. In fact, he reinstated the genus *Abel-*

Commonly used name	Correct name
Hibiscus abelmoschus Linn.	*Abelmoschus moschatus* Medik.
H. angulosus Mast.	*A. angulosus* Wt. & Arn.
H. cancellatus Roxb.	*A. cancellatus* Wall.
H. esculentus Linn.	*A. esculentus* (L.) Moench.
H. ficulneus Linn.	*A. ficulneus* (L.) Wt. & Arn.
H. manihot Linn.	*A. manihot* (L.) Medik.
H. pungens Roxb.	*A. manihot* (L.) Medik. var. *pungens* (Roxb.) Hochr.
H. tetraphyllus Roxb.	*A. manihot* (L.) Medik.
H. rugosus Mast.	*A. rugosus* Wt. & Arn.

moschus of MEDIKUS, stating that in this genus the calyx, corolla, and stamens are fused together at the base and fall as one piece after anthesis. In this paper we have followed the recent view of HOCHREUTINER and considered *Abelmoschus* (*Hibiscus pro parte*) as a genus. The common Indian species hitherto known under *Hibiscus* have, therefore, the changed names as shown above.

It will be seen from the above list that the name of the common okra, or bhendi, is now changed to *Abelmoschus esculentus* (L.) Moench.

PREVIOUS WORK ON SPECIES OF ABELMOSCHUS.—In the following review, irrespective of the nomenclatural changes indicated above, the names used by the authors of the various publications have been retained for convenience.

Besides the foregoing references, other workers (**1, 11, 15**) have also made observations on the taxonomy of species like *H. manihot*, *H. esculentus*, *H. ficulneus*, *H. abelmoschus*, and *H. hispidissimus*. Unfortunately, only brief abstracts of these papers were available for reference. Some information is also available regarding the crossing behavior of a few species. TESHIMA (**16**) observed that *H. esculentus* and *H. manihot* crossed only when the former was used as the female parent. CHIZAKI (**2**) and USTINOVA (**17, 18**) also studied this cross and reported that the F_1 hybrids were partially fertile. Crosses between *H. esculentus*, *H. abelmoschus*, and *H. manihot* were studied by SKOVSTED (**15**). In the cross *H. abelmoschus* × *H. manihot* empty seeds were obtained, whereas in the reciprocal cross seedless capsules were formed. With *H. esculentus* as female parent both *H. abelmoschus* and *H. manihot* produced viable seeds, whereas in one of the reciprocal combinations, *H. abelmoschus* × *H. esculentus*, only empty seeds were obtained. No information is given, however, as regards the fertility of the F_1 hybrids raised from viable seeds wherever obtained. SINGH *et al.* (**14**) recorded a cross between *H. esculentus* and *H. ficulneus* which formed viable seeds producing sterile F_1 hybrids.

Material and methods

During plant exploration work within the country under a scheme for the introduction of new economic plants (financed by the Indian Council of Agricultural Research) several wild species were collected: *A. ficulneus* from East Punjab, Madhya Bharat, Madhya Pradesh, and the State of Madras; *A. manihot* from Bengal, Baroda, Saharanpur (U.P.), and Jubbulpur (M.P.); *A. manihot* var. *pungens* from Dehra Dun; and *A. tuberculatus* sp. nov., described later in this paper, from Saharanpur, Ajmer, and Indore. The cultivated species *A. esculentus* was also included.

Plants were grown both in the hot weather (April sowing) and in the rainy season (July sowing). Seeds of the wild species were scarified before sowing to promote quick germination. Rows were sown $2\frac{1}{2}$ feet apart, the plants in the row being spaced 1 foot apart. In making crosses, emasculation was done on the previous evening or in the morning before the flowers opened. The anthers only were removed without disturbing the staminal column. Empty seeds were differentiated from well-filled seeds by immersion in water, the empty seeds then floating.

Experimentation and results

DESCRIPTION AND CLASSIFICATION OF VARIOUS SPECIES ON THEIR MORPHOLOGICAL CHARACTERS AND CROSSING BEHAVIOR

MORPHOLOGICAL CHARACTERS.—General descriptions of the species under

study, except *A. tuberculatus* sp. nov., were found to tally with those given in the Indian floras of the authors referred to earlier. A full description of the new species is given later. The characters of the pollen grain, which have not been given enough attention by previous workers, were found to be of interest. An account of these characters along with those of the seed is given in table 1. Various types of spines on the pollen grains are shown in figures 1–5.

TABLE 1

POLLEN GRAIN* AND SEED CHARACTERS IN ABELMOSCHUS SPECIES

Character	A. esculentus	A. tuberculatus	A. manihot var. pungens	A. manihot	A. ficulneus
Pollen Grains:					
Diameter (μ)	170.1	162.4	153.9	145.3	112.2
Length of spine (μ)	15.1	17.2	22.8	21.9	19.4
No. of spines on margin	25.5	28.9	17.0	14.9	14.7
Shape of apex of spines	Blunt and long; or short or cone-shaped	Rather acute, sometimes branched	Long, acute with broad base	Long and sharply acute	Obtuse, intermediate between *A. esculentus* and *A. manihot*
Seed:					
Shape	Round	Round	Kidney-shaped	Round	Round
Diameter (mm.)	4.52	3.41	L=3.56† B=2.83	3.57	2.97
Surface	Hairy or smooth, striated	Hairy or smooth, striated	Smooth, striated	Hairy, striated	Smooth or hairy, striated
Hilum	Hairy or glabrous	Glabrous	Glabrous	Hairy or glabrous	Hairy or glabrous

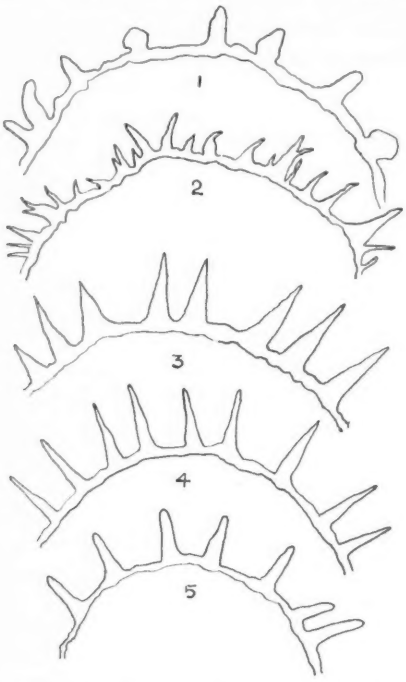
* Quantitative values given are averages of ten measurements.
† L, length; B, breadth.

The varieties of *A. ficulneus* and *A. manihot* collected from the sources referred to in this paper differed mainly in their period of flowering and fruiting and also in their growth habit. The varieties of *A. ficulneus* collected from East Punjab and Madhya Bharat were early and tall with semi-erect lower branches, whereas those from Madras and Madhya Pradesh were late and dwarf with spreading lower branches (*H. prostrata sensu* Roxburgh). In the case of *A. manihot* the varieties from Uttar Pradesh and Madhya Pradesh were early, with lower branches rather spreading, and those from Bengal and Baroda were comparatively late with ascending lower branches.

On the basis of morphological characters, therefore, it apparently is appropriate to group this material together, and the various species can be recognized as separate taxonomic units.

CROSSING BEHAVIOR.—Crosses were attempted in various combinations. The results of crossing and the nature of the F_1 hybrids are described in table 2. From these data it can be seen that *A. ficulneus* does not cross with any of the other tested species. The latter four species, however, readily cross among themselves, producing normal viable seeds. This suggests that *A. ficulneus* is not closely related to them. On the basis of the percentage of normal seeds per cap-

sule from crosses, it appears that *A. tuberculatus* is more closely related to *A. esculentus*, while *A. manihot* follows *A. tuberculatus*. *A. manihot* var. *pungens* rightly seems to be a variety of *A. manihot* as adopted by HOCHREUTINER (**8**). It



FIGS. 1–5.—Spines of pollen grains of *Abelmoschus* species. Camera lucida drawings, ×294. Fig. 1, *A. esculentus;* fig. 2, *A. tuberculatus;* fig. 3, *A. manihot* var. *pungens;* fig. 4, *A. manihot;* fig. 5, *A. ficulneus.*

is, however, interesting that in this case also the F_1 hybrids are sterile. The sterility of the F_1 hybrids of the crosses studied supports the conclusion, drawn on the basis of morphological characters, that these plants represent distinct taxonomic units.

A. TUBERCULATUS.—We also conclude that there is ample justification for the recognition of the new species *A. tuberculatus*. This material was identified as *Hibiscus esculentus* L. at the Indian Botanic Garden, Calcutta. Authorities at the Kew Botanic Gardens, to whom a referal was also made, consider that the crossing behavior indicates that the morphological differences have more than a varietal significance, and, as such, a specific rank for *A. tuberculatus* is perhaps justifiable.

A. tuberculatus resembles *A. esculentus* in several vegetative and reproductive characters. It is, however, conspicuous in having a tall habit, short internodes, rather small flowers, and profuse fruiting (fig. 6). The fruits have bristly hairs arising from tubercular bases (fig. 7). The spot at the base of the petal is not uniformly purple as it is in *A. esculentus*. The stigma is scarlet, whereas it is purple in *A. esculentus*. A Latin diagnosis and a full description of the new species is given below:

Abelmoschus tuberculatus Pal et Singh sp. nov. (Malvaceae)

Accedit ad *A. esculentum* Moench. multis in partibus, a quo tamen differt sequentibus notis: pollinis grana ornata sunt spinis tum simplicibus tum ramosis, fructuum facies prominenter tuberculatae sunt, atque petala sunt maculate purpurea ad basim; in *A. esculento* spinae sunt tantum simplices, fructuum facies sunt leves absque tuberculis, atque petala uniformiter, haud maculate, purpurea ad basim.

Annual herbaceous shrub 0.5–1 m. high in the wild state and 1.5–1.75 m. under cultivation. *Stem* terete, strigose, green with light-purple irregular patches; internodes short; branches ascending. *Leaves* alternate, petiolate; petiole 12.5–15.5 cm. long, strigose, upper surface purplish; stipules linear, about 8 mm. long, lower and middle leaves palmatipartite, lamina broadly cordate, 5–7-lobed, 10–12 cm. long and 16–18 cm. broad, lobes broadly ovate with 2–3 lobules in each, central lobe about 7 cm. long and as broad, primary veins 6–7,

uniformly diverging from the tip of the petiole, apices of lobes obtuse, margin crenulate, base deeply rounded, both surfaces hispid, upper surface more hispid than the lower. Upper leaves palmatisect, 3–5-lobed, lamina broadly ovate, about 6.5 cm. long and 8.5 cm. broad, lobes broadly lanceolate, central lobe about 8 cm. long and 2 cm. broad, primary veins 3–5, apex acute, margin denticulate, both surfaces distinctly hispid, base truncate. *Inflorescence* a raceme, 30–40 cm. long; flowers about 3.6 cm. in diameter. *Epicalyx* of 9–12 subequal bracteoles; bracteole 1 cm. long, linear, hairy, caducous. *Sepals* 5, spathaceous, circumscissile, splitting down to one side when the flower opens and falling off as one piece after anthesis; calyx tube 2.5 cm. long and 1.2 cm. in diameter. *Petals* 5, free, regular, obovate, generally yellow, sometimes white, with a basal rhomboidal purple eye. The eye contains an irregular lower half which is white. *Staminal column* creamy white; anthers reniform, yellow or white; filaments up to 1 mm. long; pollen grains with both branched and nonbranched spinules. *Carpels* 5, united; stigma scarlet, 5-lobed, lobes minutely velvety, diverging after the opening of the flower. Ovary ovoid, 8 mm. in diameter, hairy all over; style glabrous. *Fruit* capsular, 5–7.5 cm. long and 2 cm. broad, narrowly oblong with tapering, rather blunt apex; surface studded with a large number of tubercular bases from which stiff, bristly, greenish-white hairs originate; number of ridges on the fruit uniformly 5; dehiscing from top downward. *Seeds* round, black,

TABLE 2

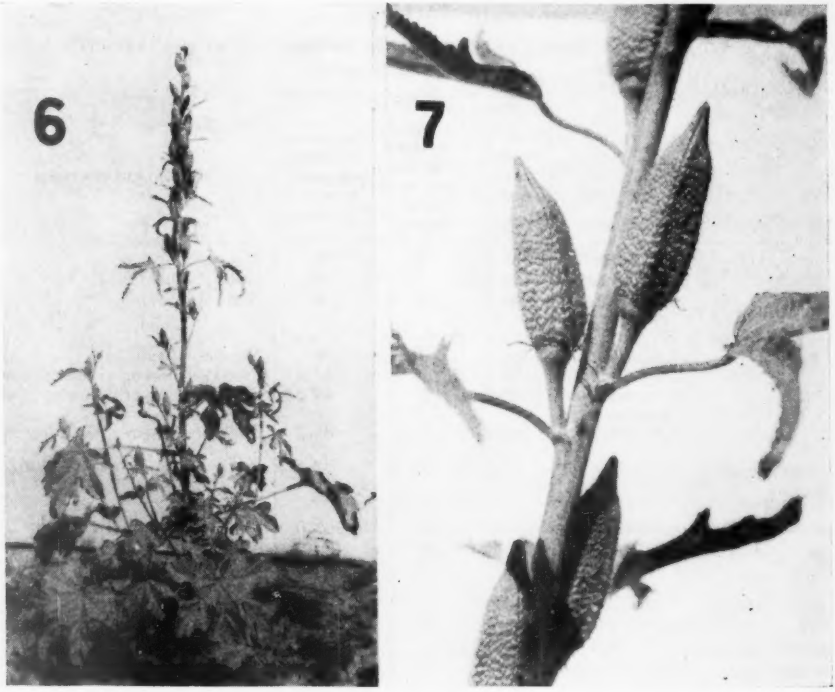
FRUIT-SETTING AND SEED DEVELOPMENT IN VARIOUS CROSSES AND FERTILITY OF RESULTING HYBRIDS

Cross		Percentage of fruit-set	Av. no. of apparently normal seeds per capsule	Percentage of viable normal seeds per capsule	Fertility of F_1 hybrids
Female parent	Male parent				
A. ficulneus	*A. manihot*	85.7	0	0	None obtained
	A. esculentus	85.7	3	0	None obtained
	A. manihot var. *pungens*	66.7	0	0	None obtained
A. manihot	*A. ficulneus*	64.3	0	0	None obtained
	A. esculentus	80.0	34.0	47.4	Fruits seedless or with empty seeds
	A. manihot var. *pungens*	40.0	2	43.3	Fruits seedless or with empty seeds
	A. tuberculatus	4.9	13	81.2	Fruits seedless or with empty seeds
A. manihot var. *pungens*	*A. manihot*	33.3	53	73.6	Fruits seedless or with empty seeds
A. esculentus	*A. manihot*	*	97	100.0	Fruits seedless or with empty seeds
	A. tuberculatus	*	89	100.0	Fruits seedless or with empty seeds
	A. ficulneus	*	0	0	None obtained
A. tuberculatus	*A. manihot*	4.9	35	88.1	Fruits seedless or with empty seeds
	A. esculentus	5.1	34	97.2	Fruits seedless or with empty seeds

* Not available.

with numerous white concentric striations all over the surface, glabrous or rarely hairy; about 3 mm. in diameter, hilum roundish, dark brown or black, glabrous.

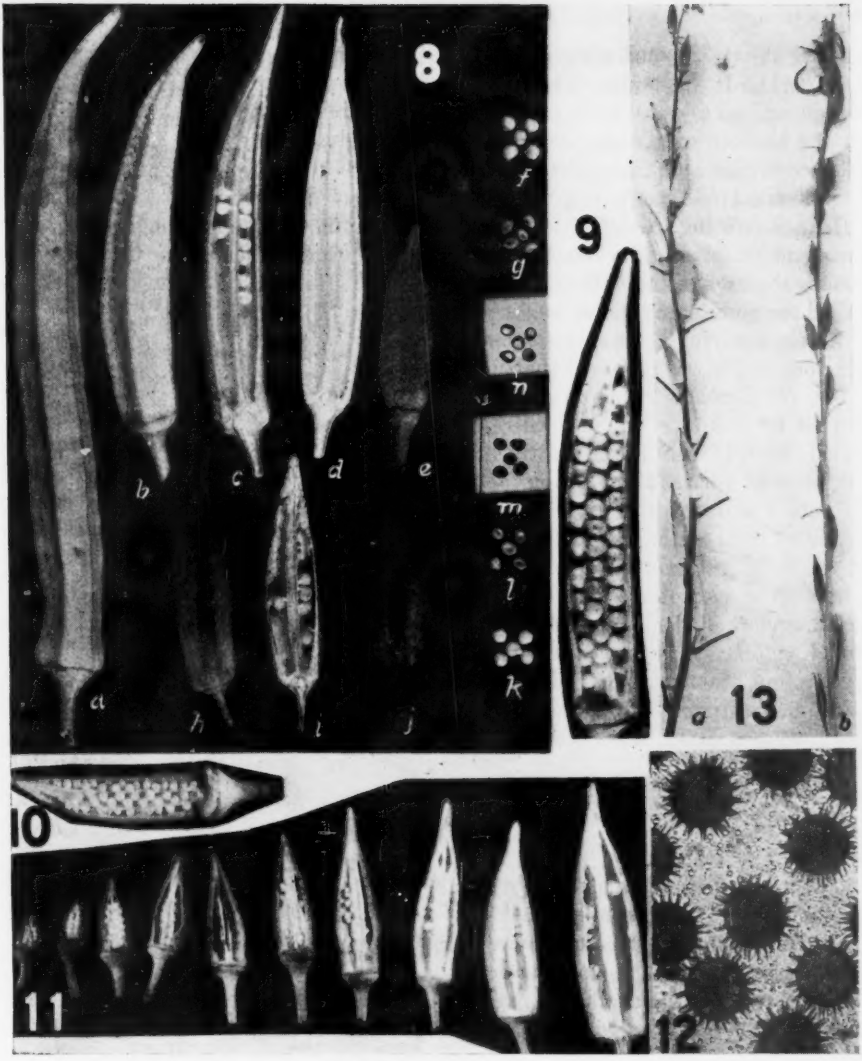
Distribution: India: Uttar Pradesh; Saharanpur, October, 1946, H. B. SINGH 950 (*Typus* in Herb. Ind. Agric. Res. Institute, New Delhi; *co-typus* in Herb. Dehra Dun, et in Herb. Kew), Lakhaoti near Bulandshahr, October, 1947, BHIM CHAND (s.n.); Ajmer Merwara, Ajmer, October, 1950, H. B. SINGH 1325; Madhya Bharat, Indore, February, 1951, H. B. SINGH 1355.



FIGS. 6–7.—*A. tuberculatus*. Fig. 6, plant showing fruits; fig. 7, part of branch showing fruits with bristly hairs arising from tubercular bases.

THE F_1 HYBRIDS

It is not proposed to present here a detailed account of the inheritance of the various vegetative and reproductive characters of the species involved in the crosses. Some of the salient points common to all the crosses, however, are given: (*a*) the hybrid plants showed strong heterosis in growth and fruiting. They were also early in flowering. (*b*) Fertility of the pollen grains varied from 40 to 95% in different crosses, the highest being in the cross *A. esculentus* × *A. manihot*. In the cross *A. esculentus* × *A. tuberculatus*, it was 35–45%. The fertile pollen grains were of two or three sizes. Fertility was greater in the rainy season than in the hot weather. (*c*) In some fruits of the F_1 hybrids there was no seed formation at all; and, in others, some apparently normal seeds formed but proved to be empty (fig. 8*c*, *d*). Ovules began to



FIGS. 8–13.—Fig. 8, fruits and seeds of parent species and F_1 hybrids: *a*, fruit of *A. esculentus;* *e*, of *A. tuberculatus;* *b*, of F_1 of *A. esculentus* × *A. tuberculatus;* *c*, longisection of fruit of same hybrid showing development of seeds; *d*, longisection of another fruit, totally seedless, of same hybrid; *f*, whole seeds, and g, transversely cut seeds, of F_1 hybrid of same cross (seeds appear normal but are empty); *f*, fruit of *A. manihot;* *h*, whole fruit, and *i*, longitudinally cut fruit, of F_1 of *A. esculentus* × *A. manihot;* *k*, whole seeds, and *l*, longitudinally cut seeds, of same hybrid (seeds are empty); *m*, whole seeds, and *n*, transversely cut seeds, of *A. tuberculatus* showing normal development. Fig. 9, longisection of well-developed fruit of *A. esculentus* showing normal seed formation. Fig. 10, longisection of fruit of *A. esculentus* 72 hours after pollination showing normal development of ovules. Fig. 11, longisection of developing fruits of *A. esculentus* × *A. manihot* showing various stages of development of ovules after pollination. Fourth from left is fruit 72 hours after pollination showing shriveling of ovules. Ultimately only a few apparently normal seeds are formed. Fig. 12, pollen of *A. manihot* showing typical spiny pollen grains of species of *Abelmoschus*. Fig. 13, *a*, fruiting branch F_1 hybrid of *A. esculentus* × *A. tuberculatus* showing profuse bearing; *b*, fruiting branch of *A. tuberculatus*.

shrivel about 72 hours after pollination (figs. 9–11). It was noticed that fruit development takes place in F_1 plants even in the absence of effective fertilization. Flowers which were emasculated but left unpollinated failed to develop into fruits. (*d*) Back-crossing to the respective parents and crossing of various F_1 hybrids among themselves and with other species failed to produce any viable seeds.

While studying the various interspecific crosses, it was observed that in the cross *H. ficulneus* × *H. esculentus* reported by SINGH *et al.* (**14**) there has clearly been an error in the identification of the wild species used as one of the parents. The description of the F_1 hybrid given by those authors agrees with that of the F_1 of *A. esculentus* × *A. manihot*. The wild species used by these authors was therefore evidently not *H. ficulneus* but *A. manihot* (*H. tetraphyllus*). *A manihot* has four bracteoles and yellow corolla with purple stigma; this agrees with the description of SINGH *et al.*

UTILIZATION OF THE WILD SPECIES AND OF THEIR HYBRIDS WITH THE CULTIVATED SPECIES

The wild species as such have not been found to be of any commercial importance for vegetable purposes. However, they possess certain useful characters which may be worth introducing into the cultivated species. The wild species, except *A. tuberculatus*, continue to flower and fruit at a time of year when it is difficult to grow the cultivated species. *A. tuberculatus* is almost immune to the attack of fruit borer (*Earias insulana*)[1] and also to the mosaic disease to which the common bhendi is susceptible. Two characters of this species, stiff hairs and thick pericarp of the fruits, are very characteristic and might be partially or wholly responsible for resistance to borers.

Analyses were made for vitamin content of the fruits of four wild species. Some of the species were found to have a vitamin C content as high, and in some cases even higher, than the cultivated species (table 3).[2] The wild species also did not show any positive reactions for alkaloids by Mayer's, picric acid, or Kraut's reagent.[3] This information is helpful because it indicates that through crossing with the wild species there is no reason to anticipate deterioration in quality with respect to vitamin C content.

TABLE 3

VITAMIN C CONTENT OF VARIOUS SPECIES OF ABELMOSCHUS (CROP SEASON OF 1948)

Species	Source	Vitamin C, mg/100 gm of fresh sample
Abelmoschus esculentus	Delhi	30.3
A. esculentus	Faizabad, U.P.	17.3
A. ficulneus	Karnal, E. Punjab	38.0
A. ficulneus	Guntur, Madras	12.0
A. manihot	Saharanpur, U.P.	7.1
A. tuberculatus	Saharanpur, U.P.	25.0

For the present the total sterility of the F_1 hybrids has caused a setback of the execution of the intended program of breeding. There was the possibility of utilizing the sterile F_1 hybrids as such, as new seedless varieties. The hybrids of *A. esculentus* with *A. tuberculatus* would have been promising for this purpose, in view of its resistance to fruit borers and

[1] Identification was kindly made by the Head of the Division of Entomology, Indian Agricultural Research Institute, New Delhi.

[2] Analyses were kindly made by the Head of the Division of Chemistry, Indian Agricultural Research Institute, New Delhi.

[3] Tests made by the Head of the Division of Chemistry, Indian Agricultural Research Institute, New Delhi.

also because of its heavy bearing (fig. 13), except that the objectionable bristly hairs on the fruits, which do not soften, even after cooking, make such fruits unattractive for culinary purposes.

Summary

1. Five species of *Abelmoschus*—*A. esculentus* (Linn.) Moench. (the cultivated species), *A. ficulneus* (L.) W. & A., *A. manihot* (L.) Medik., *A. manihot* (L.) Medik. var. *pungens* (Roxb.) Hochr., and *A. tuberculatus*—have been studied morphologically and in interspecific hybridization. These studies have confirmed the view that these species constitute a distinct taxonomic unit.

2. Results show that *A. tuberculatus* sp. nov. is more nearly related to *A. esculentus* than any other species, and *A. manihot* follows it. *A. manihot* var. *pungens* rightly appears to be a variety of *A. manihot* as adopted by HOCHREUTINER. *A. ficulneus* is, however, only distantly related. Whereas the other four species readily crossed with each other and formed normal viable seeds, crosses with *A. ficulneus* resulted in only shriveled or empty seeds.

3. The various F_1 hybrids studied were sterile; fruits were formed which were, however, either seedless or developed only a few empty seeds.

4. Backcrosses and crossing of the hybrids in various combinations failed to produce viable seeds.

5. Some useful characters of the wild species have been indicated.

6. A new species, *A. tuberculatus* Pal et Singh, is described.

We are grateful to Dr. N. PARTHASARATHY and Dr. D. CHATTERJEE of the Division of Botany for reading the manuscript and making several useful suggestions. We also acknowledge our deep indebtedness to Professor EDGAR ANDERSON of the Missouri Botanical Garden, with whom the question of the taxonomic position of the new species was discussed during his recent visit to India. We are further very grateful to Rev. H. SANTAPAU of St. Xavier's College, Bombay, for helpful suggestions regarding the nomenclature of this group of species and for the Latin diagnosis of the new species.

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LITERATURE CITED

1. CHEVALIER, A. [The origin, cultivation and uses of five *Hibiscus* species of the section *Abelmoschus*.] Rev. Bot. Appl. **20**:319–328, 402–419. 1940. (Original not seen. Pl. Breed. Abstr. **17**: no. 769.)
2. CHIZAKI, Y. [Breeding of a new interspecific hybrid between *H. esculentus* L. and *H. manihot* L.] Proc. Crop. Sci. Soc. Japan **6**:164–172. 1934. (Original not seen. Pl. Breed. Abstr. **5**: no. 528).
3. COOKE, T. The Flora of the Presidency of Bombay. Vol. 1. Taylor and Francis, London. 1903.
4. DUTHIE, J. F. Flora of the Upper Gangetic Plain. Vol. 1. Govt. of India, Printing Press, Calcutta. 1903.
5. GAMBLE, J. S. Flora of the Madras Presidency. Vol. 1. Adlard & Son, London. 1915.
6. HAINES, H. H. Botany of Bihar and Orissa. Vol. 1. Adlard & Son and W. Newman, London. 1925.
7. HOCHREUTINER, B. P. G. Revision du genre *Hibiscus*. Ann. Cons. Jard. Bot. Genève **4**:23–191. 1900.
8. ———. Genres nouveaux et discutées de la famille des Malvacées. Candollea **2**:79–90. 1924.
9. MASTERS, M. T. *In* HOOKER, J. D. Flora of British India. Vol. 1. L. Reeve & Co., London. 1875.
10. MEDIKUS, F. K. Ueber einige künstliche Geschlechter aus der Malven-Famille, denn der Klasse der Monadelphein, etc. Mannheim. 1787. (Original not seen.)
11. POPOFF, P. [Contributions to the study of okra (*Hisbiscus esculentus* L.) found in Bulgaria.] Rev. Inst. Rech. Agron. Bulg. **9**:3–15. 1939.

(Original not seen. Pl. Breed. Abstr. **12**: no. 626.)
12. PRAIN, D. Bengal Plants. Vol. **1**. W. Newman & Co., London.
13. SCHUMANN, K. *In* ENGLER and PRANTL. Die natürliche Pflanzenfamilien (ed. 1) **3** (6):47. 1895.
14. SINGH, B. N.; CHAKRAVARTI, S. C.; and KAPOOR, G. P. An interspecific *Hibiscus* hybrid between *H. ficulneus* and *H. esculentus*. Jour. Hered. **29**:37–41. 1938.
15. SKOVSTED, A. Chromosome numbers in the Malvaceae I. Jour. Genet. **31**:263–296. 1935.
16. TESHIMA, T. Genetical and cytological studies in an interspecific hybrid of *H. esculentus* L. and *H. manihot* L. Jour. Fac. Agri. Hokkaido Univ. **34**:156. 1933. (Cited by SKOVSTED [**15**].)
17. USTINOVA, E. I. Interspecific hybridization in the genus *Hibiscus*. Genetica **19**:356–366. 1937.
18. ———. A description of the interspecific hybrid of *H. esculentus* and *H. manihot*. Priroda (Nature) 1949, No. 6; 58–60. 1949. (Original not seen. Pl. Breed. Abstr. **21**: no. 728.)

ON THE STRUCTURE OF FLORAL NECTARIES

ABRAHAM FAHN

Introduction

Nectar-secreting organs of plants vary in their structure and topography as well as in the mode of secretion. The biology of nectaries has been widely studied during the nineteenth and the beginning of the twentieth century. The earlier workers on this subject concerned themselves chiefly with the morphology of nectaries. Anatomical investigation was first done by CASPARY (**4**), who showed that nectar is produced by a special tissue and not by vascular bundles as had previously been thought. In 1879 an important paper by BEHRENS (**1**) appeared in which not only detailed anatomy of the nectaries was recorded but nectaries were classified into a number of distinct types according to the mode of secretion. A different classification of nectaries, this one according to their localization in the different parts of the flower, was established by BONNIER (**2**). The present author has shown in a previous paper (**14**) that the amount of nectar secreted is correlated with the volume of the nectariferous tissue.

Other important contributions to our knowledge of the morphology and anatomy of nectaries were made by KNUTH (**15**), COOK (**5**), SCHNIEWIND-THIES (**18**), and DAUMANN (**6**–**13**).

The author believes that knowledge on the anatomy of nectaries is rather scant and that additional data are needed for greater insight on the subject. This paper is the result of anatomical examinations of floral nectaries in a large number of plant species which, as far as is known, have not been previously investigated. Anatomical descriptions of representatives of the main morphologico-topographical types, as established by BONNIER (**2**) and somewhat modified by the author, are given.

Material and methods

Examinations of a hundred and eighty plant species belonging to fifty families were made; most of the species are native to or cultivated in Palestine. Only a few of these are described.

After examination of the gross morphology freehand sections of the nectariferous tissue of fresh flowers were prepared for microscopic observation. The cuticle was stained by Sudan III, and the nectar-bearing cells identified by Fehling's solution (**2**). Some microtome sections of fixed material were also used.

Observations

Torus type

Marginal nectaries (Capparis sicula Duham.).—The outer surface of the nectary is triangular in shape, the upper angle of the triangle facing the stamens, while its base touches the base of the larger boat-shaped sepal in which nectar accumulates abundantly. Adjacent to the other sides of the triangle are the posterior petals. Their lower margins are somewhat thickened and revolute, leaving only a narrow slit between them. Above the edges of the petals overlap (fig. 1).

In order to suck nectar from the boat-shaped sepal, the insect separates the two petals with its feet and inserts its proboscis down the slit between the two thickened margins of the petals. A median section of the flower (fig. 2) shows the nectariferous tissue penetrating deeply into the receptacle. The epidermis is covered by a thin cuticle and contains numerous ellipsoid stomata (fig. 3), through which the nectar is secreted.

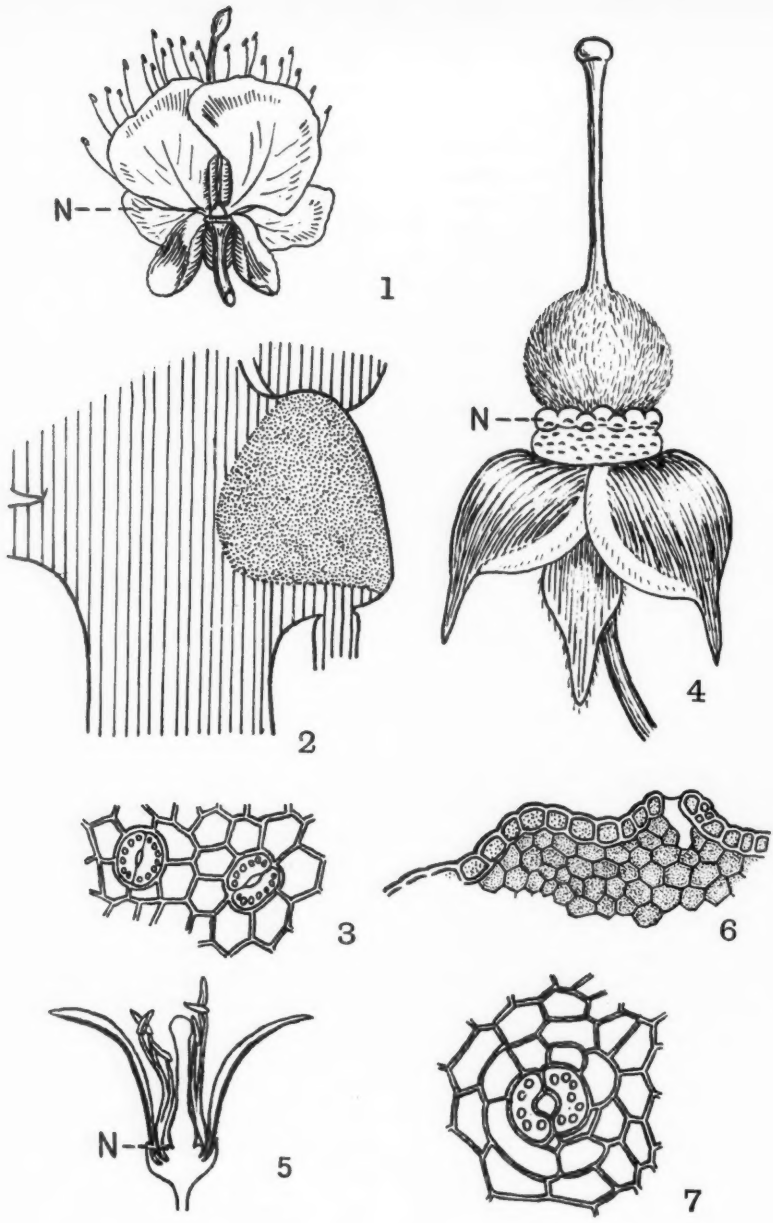
Discoid nectaries (Cistus villosus L.).—This plant, usually regarded solely as a pollen plant, is frequently visited by bees for its nectar. The receptacle next to the ovary is swollen around the bases of the stamens of the inner whorl and is transformed into tissue of the nectary (fig. 4). The epidermis contains stomata but lacks a cuticle. The mode of nectar secretion was not studied.

(Citrus limon [L.] Burmann.).—In the citrus family the nectary forms a ring round the base of the ovary (fig. 5). A transverse section of the nectary (fig. 6) shows an epidermis built of small, cubical, thick-walled cells with a thin cuticle. At the top of raised areas orbicular stomata with large openings (fig. 7) open into deep air chambers. The cells beneath the epidermis are small and crowded together. All nectary cells, including the epidermis, contain colorless granules. Occasionally crystals are found in this tissue as well as in other parts of the plant. After the nectary disk has been dried, small drops of nectar secreted through the open stomata can easily be observed with a lens.

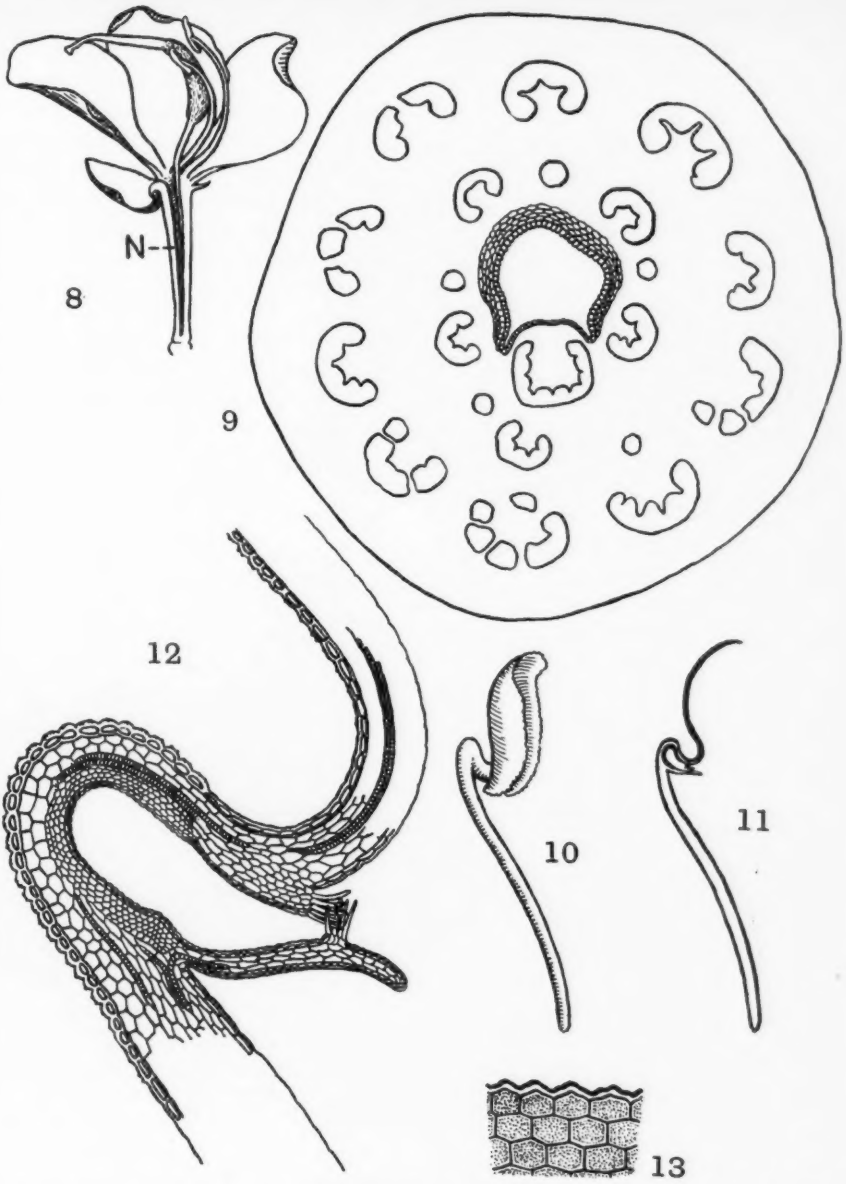
Tubular nectaries (Bauhinia purpurea L.).—In this plant the nectary tissue surrounds the inner surface of the spur which is sunken in the pedicel. The gynophore is attached to the wall of the spur (fig. 8). A transverse section of the pedicel (fig. 9) shows two whorls of vascular bundles and in the center the larger bundle of the gynophore. Near the latter the cavity of the spur is seen, surrounded by small, more or less transparent, epidermal, thin-walled cells without stomata. Inside the epidermis the nectariferous tissue is built up of small cells with yellow, granular contents. This tissue is completely lacking next to the gynophore. The nectar passes by diffusion through the thin cell walls and accumulates in the spur.

Perigonial type

Garidella unguicularis Lam.—In this plant the nectary is located in the knee of the petals where the blade passes into the claw (fig. 10). This knee forms a kind of spur, the opening of which is closed by a lid attached to the claw (fig. 11), into which nectar is secreted. Clusters of unicellular hairs near the margin of the lid almost completely close the opening of the spur. The proboscis of the insect enters the spur between these hairs. The tissue of the nectary consists of a few layers of small cells strikingly different from those adjacent. The outer wall of the epidermis is



FIGS. 1–7.—Figs. 1–3, *Capparis sicula* Duham. Fig. 1, general view of flower after sepal in which nectar accumulates has been detached; fig. 2, median section through receptacle in region of nectary; fig. 3, surface of nectary. Fig. 4, *Cistus villosus* L., flower after petals and stamens have been detached. Figs. 5–7, *Citrus limon* (L.) Burmann. Fig. 5, median longisection through flower; fig. 6, nectariferous tissue; fig. 7, surface of nectary.

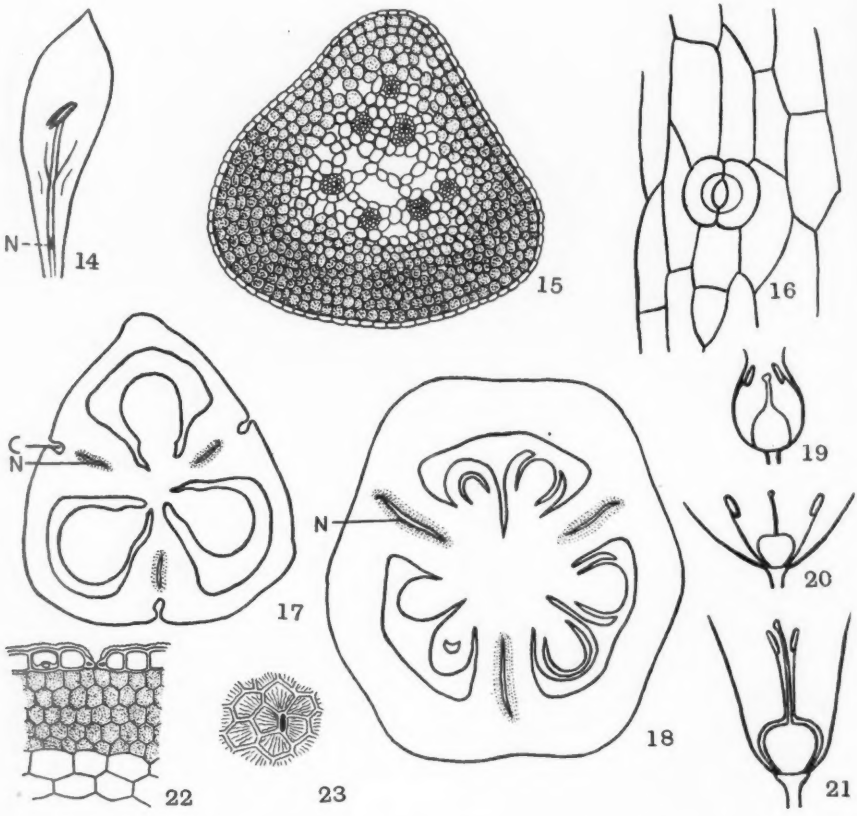


FIGS. 8–13.—Figs. 8, 9, *Bauhinia purpurea* L. Fig. 8, median longisection through flower; fig. 9, transverse section through receptacle. Figs. 10–13, *Garidella unguicularis* Lam. Fig. 10, general view of petal fig. 11, median longisection through whole petal; fig. 12, median longisection in region of nectary; fig. 13 part of nectariferous tissue.

thickened in the form of a low cap and covered with a cuticle. The content of the epidermal cells does not differ from that of other cells of the nectary (fig. 13). The

STAMEN TYPE

COLCHICUM RITCHII R. BR.—The nectariferous tissue in this species is found in the lower portions of the stamen, the



FIGS. 14–23.—Figs. 14–16, *Colchicum ritchii* R. Br. Fig. 14, general view of perianth segment with its stamen; fig. 15, transverse section of filament in region of nectary; fig. 16, surface of nectary. Figs. 17, 18, cross sections of ovaries. Fig. 17, *Muscari racemosum* (L.) Lam. et DC.; fig. 18, *Asphodeline lutea* (L.) Rchb. *N*, nectary; *C*, outer canal. Figs. 19–21, schematic longisections of flowers with septal nectaries. Fig. 19, *Muscari;* fig. 20, *Allium;* fig. 21, *Asphodeline*. Figs. 22, 23, *Bupleurum lancifolium* Hornem. Fig. 22, nectariferous tissue; fig. 23, surface of nectary.

cytoplasm of these cells is granular and yellow in color. Vascular bundles reach the nectary (fig. 12). At the time of secretion the outer walls of the epidermal cells swell, the cuticle disrupts, and the nectar is released.

base of which adheres to the perianth segments. From the outside the tissue appears as a yellow ring surrounding the basal parts of the stamens (fig. 14). On both sides of the filament a fold of the perianth is raised, and between these

the nectar is gathered. A transverse section of the nectariferous portion of the filament (fig. 15) shows transparent epidermal cells covering a layer of cells with dense granular contents and small intercellular spaces. In the center is a parenchymatous tissue with large intercellular spaces. On the side of the filament facing the petal the tissue of the nectary is denser and darker in color (brownish yellow) than on the opposite side (bright yellow). There is no recognizable cuticle over the epidermis. In the region of the nectary there are large round stomata (fig. 16) through which the nectar is secreted.

Ovarial type

Septal nectaries (Asphodelus microcarpus Viv., Asphodeline lutea [L.] Rchb., Muscari racemosum [L.] Lam. et DC.).—The nectaries in these species, as in many other members of the Liliaceae, are septal. In each of the three septa of the ovary there is a slit (figs. 17, 18) surrounded by nectariferous tissue. Nectar diffuses from this tissue into the slit and fills it. In *Asphodelus* and *Asphodeline* the slits open at the top of the ovary, and the drops of nectar percolate out. The nectar then flows into the area between the ovary and the broad bases of the filaments bent around the ovary and passes to the ovary base by capillarity (fig. 21). This capillary area is of great importance, since without it the nectar would be lost because of the horizontal or inclined position of the flower.

The above differs from the structure of the septal nectary in *Allium* and *Muscari*, where each slit is accompanied by an open canal close to the surface of the ovary and is connected with it at the top of the ovary. The nectar passes through the canal and accumulates between the base of the ovary and the perianth (*Muscari*, fig. 19) or between the former and the bases of filaments (*Allium*, fig. 20).

Thus there appears to be an obvious correlation between the shape and position of the filaments and the presence or the absence of special canals through which the nectar is poured out from the nectaries. In both types, *Allium* and *Asphodelus*, the direction of the nectar flow is the same—that is, the nectar flows from the septal slit at or near the top of the ovary and accumulates between the ovary and the filament bases. In the *Allium* type, where the filaments are straight, the nectar is conducted by particular canals; in *Asphodelus* and *Asphodeline*, which have bent filaments, canals are lacking, and the nectar is carried in the capillary space between the ovary wall and the filaments.

Stylar type

Bupleurum lancifolium Hornem.—The nectary of this plant, like that of all members of the Umbelliferae, occurs at the base of the style (stylopodium). In transverse section (fig. 22) the cells of the transparent epidermis are seen to possess thick walls and are covered by a strikingly furrowed cuticle (fig. 23). The nectar is secreted through small, narrow stomata. Beneath the epidermis there is a thick layer of closely packed, secreting cells with granular contents. These nectariferous cells are clearly differentiated from the parenchymatous cells in the layer below.

Summary and conclusions

1. Anatomical descriptions of nectaries representing the following morphologico-topographical types are given. Torus type: (*a*) marginal nectaries (*Capparis sicula* Duham.), (*b*) discoid nectaries (*Cistus villosus* L. and *Citrus limon* [L.] Burmann), (*c*) tubular nectaries

(*Bauhinia purpurea* L.). Perigonial type: *Garidella unguicularis* Lam. Stamen type: *Colchicum ritchii* R. Br. Ovarial type: septal nectaries (*Asphodelus microcarpus* Viv., *Asphodeline lutea* [L.] Rchb., *Muscari racemosum* [L.] Lam. et DC.). Stylar type: *Bupleurum lancifolium* Hornem. The examples were chosen from the total number examined: 180 species in 50 different families.

2. The structure of the nectariferous tissue is essentially similar in the nectaries of all plants investigated.

3. Different modes of secretion of nectar can be distinguished in the plants examined on the basis of the varying structure of the epidermis of the nectary: (*a*) Through swelling of the outer cell wall and rupture of the cuticle—*Garidella unguicularis* Lam. (*b*) Through stomata—*Capparis sicula* Duham., *Colchicum ritchii* R. Br., *Citrus limon* (L.) Burmann. (*c*) By diffusion through thin cell walls—*Bauhinia purpurea* L. These types correspond to those already established by BEHRENS (1).

4. Correlation was found to exist between the structure of the nectary and the shape of the filaments in *Asphodelus microcarpus* Viv. and *Asphodeline lutea* (L.) Rchb. on one hand, and in *Allium* spp. and *Muscari racemosum* (L.) Lam. et DC., on the other.

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LITERATURE CITED

1. BEHRENS, W. J. Die Nectarien der Blüten. Flora **62**:2–11, 17–27, 49–54, 81–90, 113–123, 145–153, 233–240, 241–247, 305–314, 369–375, 433–457. 1879.
2. BONNIER, G. Les Nectaires. Thèses Fac. Sci. Paris. 1879.
3. BROWN, W. H. The bearing of nectaries on the phylogeny of flowering plants. Proc. Amer. Phil. Soc. **79**:549–595. 1938.
4. CASPARY, R. De nectariis. Elverfeldae. 1848. (Cited after BEHRENS, 1.)
5. COOK, W. S. The structure of nectar glands of Iowa honey plants. Proc. Iowa Acad. Sci. **30**: 301–329. 1923.
6. DAUMANN, E. Zur Biologie der Blüte von *Nicotiana glauca* Grah. Biol. Gener. **4**, (6–8): 571–588. 1928.
7. ———. Das Blütennektarium von *Magnolia* und die Futterkoerper in der Blüte von *Calycanthus*. Planta **11**:108–116. 1930.
8. ———. Das Blütennektarium von *Nepenthes*. Beih. Bot. Centralbl. Abt. I, **47**:1–14. 1930.
9. ———. Nectarien und Bienenbesuch bei *Opuntia monacantha* Haw. Biol. Gener. **6**(3): 353–376. 1930.
10. ———. Zur Phylogenie der Diskusbildungen. Beih. Bot. Centralbl. Abt. I, **48**:183–208. 1931.
11. ———. Zur morphologischen Wertigkeit der Blütennektarien von *Laurus*. Beih. Bot. Centralbl. Abt. I, **48**:209–213. 1931.
12. ———. Zur Morphologie und Oekologie der Blüte von *Stratiotes aloides* L. Planta **14**:766–776. 1931.
13. ———. Über korkartige Substanzen im Blütennektarium von *Brownea* (*Hermesias*). Beih. Bot. Centralbl. Abt. I, **49**:710–719. 1932.
14. FAHN, A. Studies in the ecology of nectar secretion. Pal. Jour. Bot., J Series **4**:207–224. 1949.
15. KNUTH, P. Handbuch der Blütenbiologie I, II–1, II–2, III–1, III–2. Leipzig. 1898–1905.
16. NORRIS, T. Torus anatomy and nectary characteristics as phylogenetic criteria in the Rhoeadales. Amer. Jour. Bot. **28**:101–113. 1941.
17. PURI, V. The role of floral anatomy in the solution of morphological problems. Bot. Rev. **17**: 471–553. 1951.
18. SCHNIEWIND-THIES, J. Beiträge zur Kenntnis der Septalnektarien. Abstr. from Bot. Centralbl. **69–70**:216–218. 1897.

DISTRIBUTION OF THE BITTER PRINCIPLE IN THE SHOOT OF HELENIUM TENUIFOLIUM[1]

LORA MANGUM SHIELDS

Introduction

The different organs of *Helenium tenuifolium* Nutt. yield varying quantities of a colorless, crystalline bitter principle which gives an unpleasant taste to the milk of cows eating this species. Mature leaves and flowers contain the highest concentrations of this material, amounting to about 3% on a dry-weight basis (**7**). This water-soluble bitter principle has been identified as a mixture of tenulin and isotenulin, both of which have the empirical formula $C_{17}H_{22}O_5$ (**2**) and contain an α,β-unsaturated carbonyl group with one α- or one β-substituent (**7**). In addition to an acetoxyl group (**2**), isotenulin contains a group, tentatively assigned a lactone structure, which consumes one mole of alkali and which gives a positive Legal test for unsaturated lactones (**3**). The latter reaction is also characteristic of purified tenulin.

The present study was made to determine the distribution of the bitter principle in leaves, flowers, and stems of *H. tenuifolium*.

Material and methods

The plant materials were collected during August, September, and October in several counties around State College, Mississippi. They were preserved in full-strength formalin or in a saturated solution of picric acid, in both of which tenulin and its isomer are only slightly soluble. Microchemical tests were made on transverse and paradermal freehand sections from young and mature leaves, flowers, and stems.

A general test for the bitter principle is made by flooding freehand sections on a microslide with alkaline picric acid, which reacts with both tenulin and isotenulin to form an orange-red molecular complex (**8**). To make the Legal test for the unsaturated lactone group, freehand sections placed on a depression slide are covered with 1 ml. of 95% ethyl alcohol to dissolve the tenulin-isotenulin mixture. Three drops of 10% HCl and one drop of 0.5% aqueous sodium nitroprusside are added to the resulting solution, which is then neutralized with 10% NaOH, allowing an interval of 2 minutes between successive drops. When the solution is barely alkaline, aqueous sodium nitroprusside is added, one drop at each 2-minute interval (**6**). The deep red color, which appears transiently upon the first addition of NaOH, intensifies and remains without fading as successive drops of sodium nitroprusside are added. Acidification with 10% HCl at this point causes structures containing tenulin or isotenulin to assume a blue-green color.

Observations

LOCALIZATION OF BITTER PRINCIPLE.—The qualitative tests on freehand sections indicate that tenulin and its isomer occur in traces in all tissues but appear in a concentrated form only in two types of trichomes. These ordinarily grow in groups of three in depressions distributed

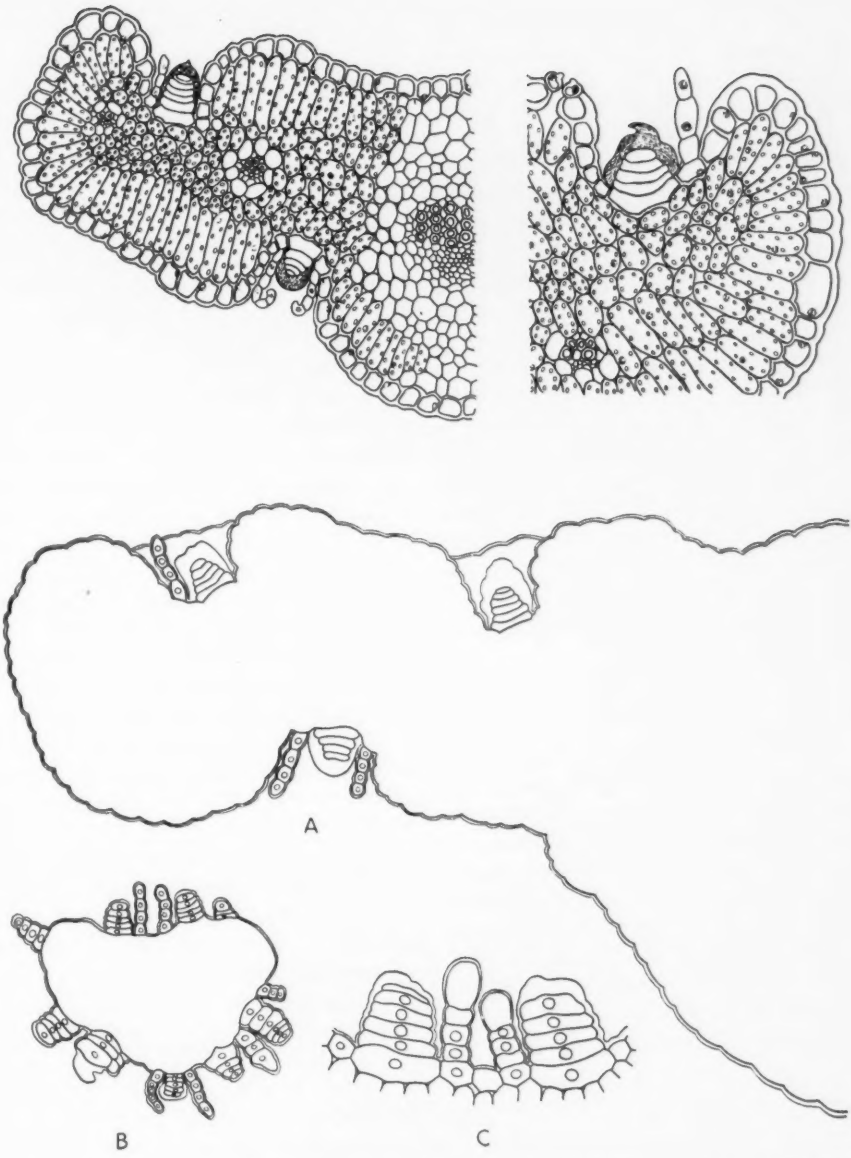
[1] The completion of this investigation was made possible by a grant from The Society of Sigma Xi.

at fairly regular intervals over both leaf surfaces at maturity, on all flower parts, and on unsuberized areas of the stem. Two slender, uniseriate hairs, each consisting of a row of four to eight isodiametric cells, grow on opposite sides of a large, glandular trichome formed by a pyramid of stratified cells loosely surrounded by a distended cuticle (fig. 1). Staining reactions with Sudan III and safranin differentiate this cuticular dome from the cellulose walls of the inclosed cells. Color tests for the bitter principle within the locally inflated cuticle are especially intense after flooding sections with 95% ethyl alcohol to dissolve the tenulin and isotenulin deposited in a solid form on the surface of the inclosed stratified cells and particularly thick at the end of the terminal cell. The color reaction is most instantaneous in terminal cells of the two lateral trichomes and on the outer face of the end cell of the large central trichome.

Individual slender, uniseriate trichomes appear sporadically on the unindented epidermis, but the trichome groups in mature parts grow only in surface depressions, and there are no depressions where such emergences are lacking. In the mature leaf the epidermal surface at the point of origin of such a group of hairs is depressed one-fourth to one-third the leaf thickness. The pits are oval and distinct, not forming grooves, and are separated from surrounding depressions by an average approximate distance of 200 μ. Four or more of these pits, usually two above and two below, are visible in every transverse freehand section of the mature leaf. Palisade cells, which elsewhere constitute the upper and lower mesophyll layers, are lacking or weakly developed adjacent to the pits (fig. 1).

Typical trichome groups appear on all flower parts except the stigma, which is covered by a dense growth of slender, uniseriate hairs only. The distribution of trichome-bearing epidermal pits on the unsuberized portions of the stem corresponds closely to that on the leaf. Concentration of the bitter principle in these specialized trichomes on the epidermal surfaces may account for the low yield of tenulin and its isomer by stems, in which the surface-volume ratio is relatively low compared with leaves and larger flower parts. No observations were made on sections from roots, which, like stems, yield relatively small amounts of tenulin and isotenulin.

ONTOGENY OF LEAF TRICHOMES CONTAINING THE BITTER PRINCIPLE.—The origin and growth of the trichome triad were traced in the developing leaf, where their pattern of ontogeny is essentially the same as in other plant parts. Each group of three trichomes originates in the leaf bud from three adjacent epidermal cells. The enlarged central trichome develops earliest, beginning with the division of a protruding epidermal cell on the unindented surface. Several successive divisions in the same plane produce a uniseriate, erect structure consisting of four to eight, wide, paradermally flattened cells (fig. 2*B*, *C*) with distinct, large nuclei and dense protoplasmic contents. Usually the outermost cells, and sometimes all cells, divide once in a plane at right angles to the leaf length, resulting in a terminally or completely biseriate trichome. As the leaf matures, the cuticular covering inclosing the glandular head thus formed becomes distended as a result of the accumulation of tenulin, isotenulin, and water between the cuticle and epidermis. Nuclei and cytoplasm of the stratified cells are gradually obscured or replaced by an accumulation of the bitter principle, which is also deposited in a crystalline form on the outer walls.



FIGS. 1, 2.—*Helenium tenuifolium*. Fig. 1, *above*, transverse sections of leaf showing depressions bearing trichome groups where bitter principle appears in concentrated form. Fig. 2, *below*, nucleated cells of trichomes approaching full size (*B*, *C*) on unindented surface of young leaf (*B*) which is less than one-eighth width of mature leaf where trichomes appear in epidermal depressions resulting from failure of palisade development in adjacent mesophyll (*A*).

Each of the two slender lateral trichomes originates by the paradermal division of an epidermal cell on one side of the developing glandular hair. By several successive divisions a structure four to eight cells in length is formed. While the terminal cells especially give intense color tests for tenulin and isotenulin, nuclei and other protoplasmic contents, including plastids, remain distinguishable, and there is no excretion of the bitter principle leading to distension of the cuticle.

The trichomes are of surface origin in the leaf primordium. Retardation of development in the mesophyll adjacent to the trichome groups results in the characteristic absence of palisade cells within the epidermis lining the surface depressions. The trichomes mature early in leaf ontogeny, some approaching full size even when the young blade has expanded to less than one-eighth its mature width (fig. 2*A*, *B*).

Discussion

Development similar to that of the large central trichome of *H. tenuifolium* occurs in the formation of oil glands of certain Compositae (1). Glandular trichomes of *Orthosiphon stamineus* Benth. secrete oil beneath the cuticle, which, as a result, becomes raised like a dome over the gland cells (5). In certain Labiatae secretion from a multicellular head accumulating between the cuticle and the underlying cellulose wall leads to an increase in cuticular surface sometimes as great as eightfold (9). The glandular trichomes of certain Indian halophytes resemble those of *H. tenuifolium* in being sunken into pits or protected by hairs and usually consist of a stalk cell bearing a multicellular head (4).

Summary

1. Microchemical tests on freehand sections from the leaves, stems, and flowers of *Helenium tenuifolium* Nutt. show the bitter principle, a mixture of tenulin and isotenulin, to be present in traces in all tissues and in a concentrated form only in two types of trichomes which appear in groups of three in surface depressions on mature plant parts.

2. Each group of three trichomes originates from three adjacent epidermal cells on the unindented surface of the organ primordium. In the mature leaf the depressions result from a retardation of palisade development adjacent to the epidermal cells bearing the trichomes.

3. Tenulin and isotenulin accumulate within the two slender lateral trichomes of each group and within and upon the outer wall surfaces of the large central trichome. The latter is formed by stratified cells loosely surrounded by a cuticle which becomes distended from the accumulation of water and tenulins in the subcuticular space.

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LITERATURE CITED

1. BRANDT, W. Über Entstehung und Funktion der Öldrusen bei Labiaten und Compositen. Proc. Internat. Congress Plant Sci., Ithaca, N.Y., 1926, 2:1362–1368. 1929.
2. CLARK, E. P. The constituents of certain species of *Helenium*. II. Tenulin. Jour. Amer. Chem. Soc. 61:1836–1840. 1939.
3. HENDLEY, E. C. The bitter principle in *Helenium tenuifolium*. Univ. of Missouri, Ph.D. thesis. 1948.
4. MULLAN, D. P. On the occurrence of glandular hairs (salt glands) on the leaves of some Indian halophytes. Jour. Indian Bot. Soc. 10:184–189. 1931.
5. MURTHY, S. N. Development of glandular hairs in *Orthosiphon stamineus* Benth. Current Sci. 10: 534–535. 1941.
6. PAIST, W. D.; BLOUT, E. R.; UHLE, F. C.; and ELDERFIELD, R. C. Studies on lactones related to

the cardiac aglycones. III. The properties of β-substituted Δα, β-butenolides and a suggested revision of the structure of the side chain of the digitalis-strophanthus aglycones. Jour. Org. Chem. 6:273–288. 1941.
7. UNGNADE, H. E., and HENDLEY, E. C. The bitter principle of *Helenium tenuifolium*. Jour. Amer. Chem. Soc. 70:3921–3924. 1948.
8. WEATHERS, N. D. Determination of the bitter substance in "bitterweed" milk. Jour. Dairy Sci. 16:401–403. 1933.
9. WENZL, H. Untersuchungen über die Exkretbildung in den Drüsenhaaren der Labiaten. Jahrb. wiss. Bot. 81:807–828. 1935.

FREE AND ACID-HYDROLYZABLE AMINO ACIDS IN FOUR RANGE GRASSES

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Introduction

The amino acid composition of the King Ranch bluestem strain of *Andropogon ischaemum* L. at various stages of development has been studied by RABIDEAU and EDWARDS (6). Earlier TSAO *et al.* (7) compared the phosphorus nutrition and nitrogen content of this strain of *A. ischaemum* L. with those of three other species: side-oats grama, *Bouteloua curtipendula* (Michx.) Torr.; sand lovegrass, *Eragrostis trichodes* (Nutt.) Wood; and Indian grass, *Sorghastrum nutans* (L.) Nash. The results of this earlier study suggested the desirability of a comparison of these four species with respect to their free and acid-hydrolyzable amino acids.

Material and methods

Plants were grown in the greenhouse in 1-gallon crocks of washed quartz sand during the fall and winter of 1950–51 by the method described by TSAO *et al.* (8). Each crock received 500 ml. of a modified Shive solution twice a week. This application of nutrient solution had been found in earlier experiments to provide an ample supply of minerals and to allow growth comparable with that obtained under field conditions.

Plants were harvested at regular intervals in the same manner as in earlier studies of RABIDEAU and EDWARDS (6). The number per sample ranged from several hundred in the case of young seedlings to a minimum of thirty in the case of older plants. Immediately after harvesting the plants were divided into roots and shoots. These samples were dried for 24 hours in a forced-air oven at 75°–85° C. The dried sample materials were then ground in a Wiley mill to pass a 60-mesh screen. Two types of amino acid determinations were made: (*a*) determination of free amino acids and the amides, glutamine and asparagine, and (*b*) determination of amino acids after acid hydrolysis. The procedure for obtaining the extracts and making chromatograms was that described by RABIDEAU and EDWARDS (6). As in this earlier work, DENT's (2) visual method was employed for the estimation of amino acid concentrations using suitable standards prepared from pure amino acids. Ordinarily 5-μgm. samples were chromatographed, but special 10-, 25-, and 50-μgm. samples were employed to detect amino acids in low concentration.

Results

Distributions of both free amino acids and those derived from acid hydrolysis of duplicate samples of shoots of each of the species at progressive ages are shown in table 1. The figures are per-

centage values based on the dry weights of the samples. In all instances the amounts of free amino acids constitute but small fractions of the amounts of total amino acids detectable after acid hydrolysis. The amounts of individual free amino acids were frequently so small as to make their detection very difficult. Although the figures for free amino acids are not altogether consistent, probably in part because of the difficulty in detecting such small amounts of these amino acids, there does appear to be a general decrease in the percentage of free amino acid present in the shoots as the age of the plants increases. Some differences among the species in free amino acid content are apparent. Calculation shows that *A. ischaemum* and *B. curtipendula* generally had the highest free amino acid contents, followed by *E. trichodes* and *S. nutans*. *B. curtipendula* had more leucine, valine, asparagine, and aspartic acid than did *A. ischaemum*, but *A. ischaemum* contained more alanine and glutamic acid than did *B. curtipendula*. *S. nutans* had a higher content of glutamine, asparagine, lysine, glutamic acid, aspartic acid, and valine than did *E. trichodes*, but the latter had more leucine, alanine, and methionine than did *S. nutans*. *S. nutans* had the highest content of glutamine, and *E. trichodes* had the highest content of alanine and methionine. Because of the difficulty in assaying such small amounts of these free amino acids by the method employed, further investigation would be required to establish with certainty such differences as are here indicated.

Figures on amino acids derived from acid hydrolysis of shoot samples are also given in table 1. These values are uncorrected for the free amino acids present. This error is not large, however, since the amount of detectable free amino acids was, in all but a few instances, fairly small. These data appear to indicate a general decrease in the percentage of acid-hydrolyzable amino acids of the shoots with increase in age of the plants. Calculations show that *B. curtipendula* had the highest acid-hydrolyzable amino acid content and was followed, in turn, by *E. trichodes*, *S. nutans*, and *A. ischaemum*. *E. trichodes* contained the most valine, alanine, histidine, and methionine, but *B. curtipendula* had only slightly smaller amounts of these amino acids. *S. nutans* exceeded *E. trichodes* with respect to glutamic acid only. With regard to the percentage concentration of these amino acids, *A. ischaemum* had the lowest content of all four species. The various amino acids derived by acid hydrolysis were found consistently, although in varying amounts, in essentially all of the samples regardless of the age of the plants. This is in contrast to the distribution of the free amino acids where lysine, arginine, proline, serine, histidine, and threonine were detected at only one or two of the sampling periods for any of the species. Glutamine and asparagine were detected in some of the free amino acid extracts but were not detected after acid hydrolysis, presumably since acid hydrolysis had converted these amides to the corresponding amino acids. Root samples (not shown in table 1) of these same four species gave evidence of a content of free and acid-hydrolyzable amino acids similar to that in the shoot samples. Total nitrogen percentages calculated from total percentages of amino acids detected by chromatography of acid-hydrolyzed shoot samples (table 1) are shown in table 2.

Discussion

The existence of free amino acids in grasses has been well established by

TABLE 1

AMINO ACID CONTENT OF SHOOTS OF A. ISCHAEMUM, B. CURTIPENDULA, E. TRICHODES, AND S. NUTANS AT VARIOUS AGES

Figures are percentage values based on dry weights. Figure before slant line represents percentage of free amino acid; figure after slant line represents percentage of amino acid in acid hydrolysate of sample. Latter values are uncorrected for free amino acid occurring in samples prior to acid hydrolysis.

AMINO ACID	SPECIES	AGE OF PLANT IN DAYS									
		Seed	3	8	15	21	28	37	43	51	64
Leucine	*B. curtipendula*	0.1/5.0	0.8/3.6	0.8/4.0	0.0/4.0	0.2/3.6	0.3/5.0	0.2/5.0	0.2/3.6	0.2/3.6	0.2/3.6
	E. trichodes	0.0/4.0	0.2/3.2	0.1/3.6	0.2/4.0	0.2/5.0	0.7/5.0	0.1/5.0	0.2/4.0	0.2/3.0	0.0/3.6
	S. nutans	0.0/3.4	0.0/4.0	0.5/3.4	0.2/3.4	0.2/3.4	0.2/4.0	0.2/3.4	0.0/4.0	0.2/3.2	0.1/3.4
	A. ischaemum	0.0/6.0	0.0/0.0	0.5/2.7	0.8/2.7	0.3/2.7	0.5/1.0	0.0/1.5	0.1/1.0	0.2/1.5	0.2/2.0
Valine	*B. curtipendula*	0.1/3.0	0.6/2.6	0.5/2.2	0.0/2.6	0.0/2.4	0.2/3.0	0.2/2.6	0.1/2.4	0.1/2.4	0.1/2.6
	E. trichodes	0.0/2.8	0.1/2.0	0.1/2.4	0.2/2.8	0.1/3.0	0.2/2.8	0.1/2.8	0.1/2.8	0.1/2.0	0.0/2.8
	S. nutans	0.0/2.4	0.0/2.4	0.3/2.4	0.1/2.6	0.1/2.4	0.1/2.6	0.1/2.0	0.0/2.4	0.1/2.4	0.1/2.4
	A. ischaemum	0.0/3.0	0.0/0.0	0.5/1.5	0.5/1.5	0.2/1.5	0.2/0.5	0.0/0.5	Tr./0.5	0.2/0.9	0.2/1.3
Alanine	*B. curtipendula*	0.1/3.2	0.6/3.2	0.7/3.2	0.2/3.2	0.2/3.0	0.2/3.2	0.2/3.2	0.1/2.6	Tr./2.6	Tr./3.0
	E. trichodes	0.1/3.4	0.4/3.0	0.0/3.0	0.8/3.4	0.1/3.4	0.8/3.4	0.1/3.0	0.2/3.4	0.1/3.0	0.1/2.4
	S. nutans	0.1/2.4	0.1/3.4	0.9/3.4	0.3/3.4	0.2/2.4	0.3/2.4	0.2/2.0	0.0/3.0	0.2/2.4	0.1/2.4
	A. ischaemum	Tr./3.2	0.0/0.0	0.8/1.8	1.0/1.8	0.6/1.8	0.6/1.0	0.2/1.2	0.1/1.0	0.2/1.4	0.1/1.5
Threonine	*B. curtipendula*	0.0/2.0	0.3/2.0	0.1/1.0	0.0/1.0	0.0/1.0	0.0/2.0	0.0/0.5	0.0/0.5	0.0/0.5	0.0/0.6
	E. trichodes	0.0/0.5	0.0/0.5	0.0/1.0	0.0/2.0	0.0/2.0	0.1/1.0	0.0/0.5	0.0/1.0	0.0/0.3	0.0/0.3
	S. nutans	0.0/0.5	0.0/0.5	0.2/1.0	0.0/2.0	0.0/0.5	0.0/0.4	0.0/0.4	0.0/0.4	0.0/0.4	0.0/0.4
	A. ischaemum	0.0/1.0	0.0/0.0	0.1/1.0	0.1/1.0	0.0/1.0	0.0/0.0	0.0/0.0	0.0/0.2	0.0/0.7	0.0/0.6
Histidine	*B. curtipendula*	0.0/5.0	0.0/2.0	0.0/4.0	0.0/4.0	0.0/5.0	0.0/4.0	0.0/5.0	0.0/4.0	0.0/2.0	0.0/0.0
	E. trichodes	0.0/4.0	0.0/5.0	0.0/4.0	0.0/4.0	0.0/4.0	0.0/5.0	0.0/4.0	0.0/4.0	0.0/3.0	0.0/3.0
	S. nutans	0.0/4.0	0.0/4.0	0.5/0.0	0.0/0.0	0.0/4.0	0.0/4.0	0.0/4.0	0.0/4.0	0.0/0.0	0.0/4.0
	A. ischaemum	0.0/3.0	0.0/0.0	0.0/4.0	0.0/2.0	0.0/2.5	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
Serine	*B. curtipendula*	0.0/6.0	0.0/4.0	0.0/3.4	0.0/5.0	0.0/3.4	0.0/5.0	0.0/4.0	0.0/3.4	0.0/3.0	0.0/3.0
	E. trichodes	0.0/6.0	0.0/3.4	0.0/4.0	0.0/4.0	0.0/4.0	0.1/4.0	0.0/4.0	Tr./3.0	0.0/3.4	0.0/2.4
	S. nutans	0.0/4.0	0.0/4.0	0.0/3.4	0.0/4.0	0.0/3.0	0.0/3.0	0.0/3.4	0.0/3.4	0.0/3.0	0.0/3.0
	A. ischaemum	0.0/3.2	0.0/0.0	0.0/4.0	0.0/2.5	0.0/2.5	0.0/0.0	0.0/0.0	0.0/0.0	0.0/1.5	0.0/1.8
Aspartic acid	*B. curtipendula*	0.1/3.0	0.8/4.0	0.6/3.0	0.2/3.0	0.2/2.5	0.2/3.0	0.1/2.4	Tr./2.4	Tr./2.0	Tr./2.4
	E. trichodes	0.1/1.0	0.2/2.4	0.1/3.0	0.2/3.0	0.2/3.0	0.2/2.0	Tr./2.4	Tr./2.0	Tr./1.0	Tr./2.0
	S. nutans	0.1/1.0	0.1/3.0	0.7/3.0	0.2/2.5	0.1/2.0	0.1/2.0	Tr./2.5	0.0/2.0	Tr./2.0	Tr./2.0
	A. ischaemum	Tr./2.4	0.0/0.0	0.5/2.5	0.3/1.5	0.1/1.5	0.1/0.2	Tr./0.2	0.1/0.2	Tr./0.2	Tr./0.2
Glutamic acid	*B. curtipendula*	0.1/3.2	0.6/3.2	0.7/3.2	0.6/3.2	0.5/3.0	0.5/3.2	Tr./3.2	0.1/3.0	Tr./3.0	Tr./3.0
	E. trichodes	0.1/2.4	0.3/3.0	0.0/3.2	0.0/3.5	0.5/3.5	0.6/3.2	0.2/3.2	0.1/3.0	0.2/3.0	0.2/2.0
	S. nutans	0.5/2.4	0.1/3.4	0.8/3.4	0.5/3.4	0.3/3.0	0.3/3.0	0.0/3.0	0.0/3.0	0.1/3.0	0.1/2.4
	A. ischaemum	Tr./3.0	0.0/0.0	0.8/1.6	0.8/1.8	0.5/1.8	0.5/2.0	0.2/1.7	0.5/1.7	0.2/1.5	0.5/1.6
Lysine	*B. curtipendula*	0.1/5.0	0.3/3.0	0.7/5.0	0.0/3.0	0.0/2.0	0.0/7.0	0.0/6.0	0.0/4.0	0.0/4.0	0.0/2.0
	E. trichodes	0.0/3.0	0.0/2.0	0.0/5.0	0.0/4.0	0.0/6.0	0.0/4.0	0.0/4.0	0.0/5.0	0.0/4.0	0.0/3.0
	S. nutans	0.0/2.0	0.0/3.0	0.5/3.0	0.0/4.0	0.0/3.0	0.0/4.0	0.0/4.0	0.0/3.0	0.0/2.0	0.0/4.0
	A. ischaemum	0.0/6.0	0.0/0.0	0.5/3.0	0.5/3.0	0.3/3.0	0.2/1.5	0.0/1.5	0.0/2.2	0.0/2.0	0.0/1.6
Arginine	*B. curtipendula*	0.0/6.0	0.3/5.0	0.7/1.0	0.0/3.0	0.0/2.0	0.0/4.0	0.0/3.0	0.0/2.0	0.0/3.0	0.0/2.0
	E. trichodes	0.0/2.0	0.0/1.0	0.0/5.0	0.0/2.0	0.0/5.0	0.0/4.0	0.0/2.0	0.0/4.0	0.0/2.0	0.0/3.0
	S. nutans	0.0/2.0	0.0/2.0	0.0/2.0	0.0/2.0	0.0/2.0	0.0/4.0	0.0/2.0	0.0/5.0	0.0/2.0	0.0/3.0
	A. ischaemum	0.0/6.0	0.0/0.0	0.2/3.0	0.3/2.0	0.0/2.5	0.0/0.0	0.0/0.0	0.0/1.3	0.0/0.0	0.0/0.0
Proline	*B. curtipendula*	0.0/5.0	0.0/2.0	0.0/2.0	0.0/1.0	0.0/2.0	0.0/2.0	0.0/4.0	0.0/2.0	0.0/1.0	0.0/1.0
	E. trichodes	0.0/5.0	0.0/1.0	0.0/0.0	0.0/3.0	0.0/2.0	0.0/4.0	0.0/4.0	0.0/1.0	0.0/1.0	0.0/0.0
	S. nutans	0.0/1.0	0.0/1.0	0.0/3.0	0.0/0.0	0.0/2.0	0.0/2.0	0.0/1.0	0.0/1.0	0.1/3.0	0.0/1.0
	A. ischaemum	0.0/3.0	0.0/0.0	0.0/2.0	0.0/2.0	0.0/1.5	0.0/1.5	0.0/1.5	0.0/1.3	0.0/1.5	0.0/1.1
Methionine	*B. curtipendula*	0.1/3.0	0.8/1.0	0.8/2.0	0.0/2.0	0.0/0.0	0.0/2.0	0.0/1.0	0.0/1.0	0.1/1.0	0.1/0.0
	E. trichodes	0.0/2.0	0.3/2.0	0.0/2.0	0.7/2.4	0.0/1.0	0.7/2.0	0.1/1.0	0.2/1.0	0.0/0.0	0.0/0.0
	S. nutans	0.0/2.0	0.0/0.0	0.4/0.0	0.0/0.0	0.0/0.0	0.0/1.0	0.1/1.0	0.0/1.0	0.2/0.0	0.0/0.0
	A. ischaemum	0.0/0.0	0.0/0.0	0.5/0.0	0.6/0.0	0.2/0.0	0.2/0.0	0.0/0.0	0.0/0.0	0.2/0.0	0.0/0.0
Asparagine	*B curtipendula*	0.1/0.0	0.8/0.0	1.2/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.1/0.0	0.1/0.1	0.1/0.0
	E. trichodes	0.0/0.0	0.0/0.0	0.1/0.0	0.2/0.0	0.0/0.0	0.2/0.0	0.1/0.0	0.1/0.0	Tr./0.0	Tr./0.0
	S. nutans	0.0/0.0	0.0/0.0	0.7/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.1/0.0	0.0/0.0	0.1/0.0	Tr./0.0
	A. ischaemum	0.0/0.0	0.0/0.0	1.3/0.0	0.5/0.0	0.3/0.0	0.1/0.0	0.0/0.0	0.0/0.0	0.1/0.0	0.1/0.0
Glutamine	*B. curtipendula*	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.1/0.0	0.1/0.0	0.1/0.0
	E. trichodes	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	Tr./0.0	0.0/0.0	Tr./0.0	Tr./0.0	Tr./0.0
	S. nutans	0.0/0.0	0.0/0.0	0.1/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
	A. ischaemum	0.0/0.0	0.0/0.0	Tr./0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0

CHIBNALL (1) and by LUGG (5). The present work indicates that the percentage content of a water-soluble free amino acid may not bear any direct relation to the total amount of that particular acid detectable in an acid digest of similar plant samples. In *B. curtipendula*, for example, serine was not detected as a free amino acid, but upon acid hydrolysis serine was found to compose an estimated 3–6% of the dry weight of the plant tissue. In general, the percentage of either free amino acids or acids yielded by acid hydrolysis was higher in younger plants than in older plants, an observation already reported for *A. ischaemum* (6). This is in line with the earlier finding by other workers (3, 4) that total nitrogen percentage tends to decrease with age in range grasses. TSAO *et al.* (7) failed to find any consistent decrease in total nitrogen percentage with advancing age in the four species of grass used in this experiment. The conditions of the experiment conducted by TSAO *et al.* were, however, quite different from those of the present study and from the growth and collection intervals of the FUDGE and FRAPS (4) samples. The calculated nitrogen percentages shown in table 2 fall within the range of the nitrogen content of other grasses observed by FUDGE and FRAPS (4). The data of these analyses suggest that the amino acid composition of the four grasses studied differs slightly with respect both to the quantity of any one amino acid and to its relative concentration at various stages in the development of the plants. In general, however, the amino acid content is the same in all four species. The regularity of occurrence of the same amino acids in all the species at successive stages in development suggests, as has been indicated by RABIDEAU and EDWARDS (6), that the protein content of these four species may be essentially the same.

TABLE 2

PERCENTAGE NITROGEN (BEFORE SLANT LINE) DERIVED FROM DIVIDING TOTAL OF ALL AMINO ACIDS (AFTER SLANT LINE) DETECTED BY CHROMATOGRAPHY OF ACID-HYDROLYZED SHOOT SAMPLES (SEE TABLE 1) BY FACTOR 6.06

SPECIES	AGE IN DAYS									
	Seed	3	8	15	21	28	37	43	51	64
B. curtipendula	8.2/49.5	4.2/35.6	5.6/34.0	5.8/35.0	4.9/29.9	7.2/43.4	6.6/39.9	5.1/30.9	4.5/27.3	3.8/23.2
E. trichodes	6.5/39.0	4.7/28.5	6.0/36.2	6.3/38.1	7.3/44.2	6.7/40.4	5.9/35.9	5.7/34.2	4.3/25.7	4.0/24.5
S. nutans	4.5/27.0	5.1/30.7	4.6/28.0	4.5/27.3	4.6/27.7	5.2/31.4	4.7/28.7	5.2/32.2	3.9/23.4	4.6/28.0
A. ischaemum	6.6/39.8	.....	5.0/31.1	3.6/21.8	3.6/21.8	1.3/7.6	1.3/8.1	1.6/9.4	1.9/11.2	1.9/11.7

Summary

1. Free amino acids and amino acids yielded by acid hydrolysis were studied in *Andropogon ischaemum*, *Bouteloua curtipendula*, *Eragrostis trichodes*, and *Sorghastrum nutans* by a paper chromatography method.

2. The data indicate essentially the same amino acid content for all four species but with some variation from one developmental stage to another in the concentration of particular amino acids.

3. A general decrease in percentage of amino acids present appears to accompany aging of the plants. Differences in quantity of particular amino acids at given ages are set forth in a table.

The seeds used in these experiments were furnished by the San Antonio nursery of the Soil Conservation Service of

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LITERATURE CITED

1. CHIBNALL, A. C. Protein Metabolism in the Plant. Yale University Press, New Haven, Conn. 1939.
2. DENT, C. E. The amino aciduria in Fanconi Syndrome. A study making extensive use of techniques based on paper partition chromatography. Biochem. Jour. **41**:240–253. 1947.
3. FRAPS, G. S., and FUDGE, J. F. The chemical composition of forage grasses of the East Texas Timber Country. Texas Agr. Exp. Sta. Bull. 582. 1940.
4. FUDGE, J. F., and FRAPS, G. S. The chemical composition of grasses of northwest Texas as related to soils and to requirements for range cattle. Texas Agr. Exp. Sta. Bull. 669. 1945.
5. LUGG, J. W. H. The amide, tyrosine and tryptophan contents and the sulfur distributions (cystine plus cysteine and methionine contents) of some plant leaf protein preparations. Biochem. Jour. **32**:2123–2128. 1938.
6. RABIDEAU, G. S., and EDWARDS, M. B. Nitrogen and amino acid content of the various parts of *Andropogon ischaemum* L. Pl. Physiol. **26**: 798–806. 1951.
7. TSAO, T.; RABIDEAU, G. S.; and WHALEY, W. G. Effects of differences in phosphorus nutrition on the nitrogen content and phosphorus 32 absorption of four species of grass. BOT. GAZ. **112**:214–221. 1950.
8. ———, ———, and ———. The phosphorus uptake of *Andropogon ischaemum* L. at various stages of development. Plant Physiol. **25**:653–665. 1950.

INFLUENCE OF TEMPERATURE ON FORMATIVE RESPONSE OF BEAN SEEDLINGS TO 2,4-DICHLOROPHENOXYACETIC ACID[1]

JAMES W. BROWN AND ROBERT L. WEINTRAUB

Introduction

The influence of temperature on the herbicidal effectiveness of 2,4-dichlorophenoxyacetic acid (2,4-D) was recognized in the earliest publications describing such uses (**2, 3, 6**). While these and other studies concerned more specifically with the influence of temperature (**4, 5**) have been unanimous in reporting that plant response is diminished or delayed at low temperatures, no investigation seems to have been made correlating temperature specifically with the action of the growth-regulator as distinguished from indirect effects on translocation and growth in general. Such an analysis is reported here of the influence of temperature on the repression of leaf expansion owing to treatment with 2,4-D. This response appears to correlate well with herbicidal effectiveness and affords a much more quantitative measure (**1**), since, by ap-

[1] An account of these results was presented at the Columbus meeting of the Botanical Society of America, September 12, 1950.

plying 2,4-D directly upon the responsive organ, translocation is eliminated as a complicating factor. Environmental conditions have also been more closely controlled than in the previous studies.

Material and methods

Seedlings of the common bean, *Phaseolus vulgaris* L., var. Black Valentine, were cultured in a soil-sand-peat mixture in the greenhouse to the stage just prior to unfolding of the first trifoliolate leaf. Applications of 2,4-D were made, and the plants were transferred at once to an artificially lighted, temperature- and humidity-controlled room where they were allowed to develop. A 14-hour daily photoperiod was provided by overhead banks of Daylight fluorescent lamps giving an intensity of approximately 800 foot-candles at the level of the primary leaves. Temperature was held to $\pm 0.5°$ C. and relative humidity to $\pm 10\%$. Water was supplied by subirrigation.

The 2,4-D was applied on the terminal bud as a 0.005-ml. drop of alcoholic solution containing 1% Tween-20, as described elsewhere (1). Repression of expansion of the first trifoliolate leaf was calculated from measurements of leaf area. Eighteen to twenty-four plants were used per treatment in each experiment, and the experiments were repeated two or three times. In several experiments half the population was harvested for leaf measurement 7 days after application, and the other half after a longer period, determined by the temperature.

Results

RELATION OF LEAF EXPANSION TO TEMPERATURE.—Preliminary experiments on the influence of temperature (in the range 22°–34° C.) on development of untreated bean plants gave the following results. The growth curve of the first trifoliolate leaf is of the common sigmoid form. At the optimal temperature the initial phase of relatively slow expansion occupies approximately 4 days from the beginning of unfolding of the bud. The growth rate is virtually constant during the following 3–4 days and thereafter diminishes until the maximal area is attained at about 12–13 days. At temperatures above or below the optimum the lag period is extended 1–2 days; the *duration* of the linear phase is relatively unaffected, although the *rate* of growth may be greatly reduced. Consequently, although expansion may continue for a few days longer at infra- or supra-optimal temperatures, the final size attained by the leaf is less than that reached at the optimum temperature.

Comparisons of growth rates of the first trifoliolate leaf at different temperatures were conveniently made from measurements of area on the seventh day after commencement of unfolding, as this point falls in the linear portions of the curves for all temperatures in the range studied. Rates calculated in this manner doubtless represent the integration of various phases in leaf growth but are adequate for the present purpose. Figure 1 shows the relation between temperature and leaf expansion in 7 days. A rather sharp maximum occurs in the vicinity of 31° C. Curves for longer time periods possess less pronounced maxima because of the more prolonged growth at the extreme temperatures, but their general shape remains similar to that illustrated.

RESPONSE TO 2,4-D.—Application of 2,4-D to the bud just prior to its unfolding results in repression of expansion of the leaf. Its rate of growth and its final size are both diminished, but the temporal course of expansion is relatively unchanged. The degree of repression is proportional to the logarithm of the ap-

plied dose over a fairly wide interval (1).

The percentage repression of treated leaves, in comparison with controls at the corresponding temperatures, is virtually constant over the entire temperature range (fig. 2). The slightly lower effect at

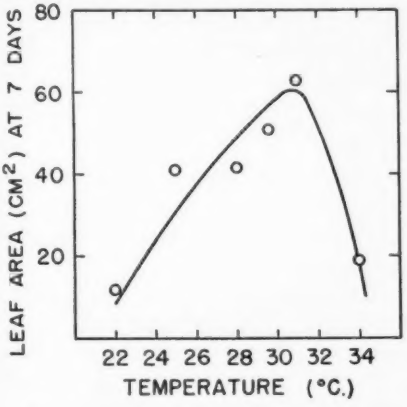

FIG. 1.—Influence of temperature on expansion of first trifoliolate leaf of Black Valentine bean.

22° C. is probably due to the greater experimental error involved in measuring the much smaller leaves produced at this temperature. Quite similar results have been obtained also with dosages both higher and lower than that illustrated. In other words, growth is repressed to the same proportionate extent at all temperatures, indicating that no temperature-limited process is involved in the inhibition itself.

Summary

1. Expansion of the first trifoliolate leaf of the bean seedling (*Phaseolus vulgaris* L., var. Black Valentine) exhibits a sharp temperature optimum at approximately 31° C.

2. Expansion is repressed by application of 2,4-dichlorophenoxyacetic acid (2,4-D) directly to the terminal bud; the degree of repression is proportional to the logarithm of the dose.

3. The percentage repression owing to a particular dose is constant over the range 22°–34° C., indicating that no temperature-limited process is involved in the inhibition.

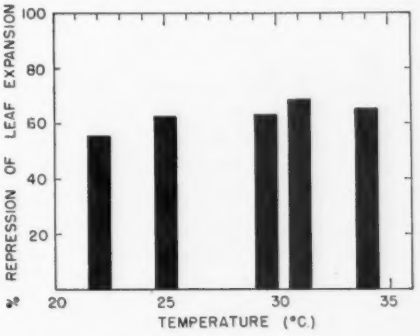

FIG. 2.—Influence of temperature on repression of first trifoliolate leaf resulting from application of 0.025 μgm. of 2,4-D directly to terminal bud.

It is a pleasure to acknowledge the technical assistance of J. ARTHUR THRONE, JEAN NICKERSON, and KATHARINE TAYLOR in this study.

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LITERATURE CITED

1. BROWN, J. W., and WEINTRAUB, R. L. A leaf-repression method for evaluation of formative activity of plant growth-regulating chemicals. BOT. GAZ. 111:448–456. 1950.
2. HAMNER, C. L., and TUKEY, H. B. The herbicidal action of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid on bindweed. Science 100:154–155. 1944.
3. ——— and ———. Selective herbicidal action of midsummer and fall applications of 2,4-dichlorophenoxyacetic acid. BOT. GAZ. 106:232–245. 1944.

4. KELLY, SALLY. The effect of temperature on the susceptibility of plants to 2,4-D. Plant Physiol. 24:534–536. 1949.
5. MARTH, P. C., and DAVIS, F. F. Relation of temperature to the selective herbicidal effects of 2,4-dichlorophenoxyacetic acid. BOT. GAZ. 106: 463–472. 1945.
6. MITCHELL, J. W.; DAVIS, F. F.; and MARTH, P. C. Turf and weed control with plant growth regulators. GOLFDOM 18:34–36. 1944.

LACK OF EFFECT OF PLANT GROWTH-REGULATORS ON THE ACTION OF ALPHA-AMYLASE SECRETED BY VIRUS TUMOR TISSUE[1]

M. K. BRAKKE AND L. G. NICKELL

Introduction

This report deals with the effect of certain synthetic plant growth-regulators on the activity of alpha-amylase (1) secreted by virus tumor tissue grown *in vitro*. Previous studies have lacked adequately controlled conditions and have been carried out with enzyme preparations obtained through the usual methods of destruction of cells. In this particular study (*a*) the assay employed to measure enzyme activity includes control of pH and temperature and (*b*) this enzyme, being secreted, is not subject to the drastic preparative procedures involving destruction of cellular structure usually necessary to obtain enzymes.

Material and methods

The tumor tissue used was induced by the wound-tumor virus (*Aureogenus magnivena* Black) in the roots of the sorrel plant (*Rumex acetosa* L.) and was the same R_1 isolate used in other studies (2, 7–9). Enzyme activity was assayed (1) by following spectrophotometrically the decrease in optical density of the starch-iodine complex. This method is sensitive to low levels of amylase, but, because of the many factors affecting the color of the complex, the reproducibility is only about $\pm 5\%$. The growth-regulators tested and the concentrations used are given in table 1.

Three different enzyme preparations consisting of unpurified spent liquid medium no. 24 (2) were used. The medium in each case had been inoculated with approximately one-tenth its weight of tumors 2–10 days earlier. The medium was used immediately after the tumors were removed and after storage overnight in the refrigerator. Enzyme activity was assayed in 1% soluble starch and 0.02 *M*, pH 4.6, acetate buffer at 40° C. Growth-regulator solutions were adjusted to pH 4.6 before being added to the reaction mixture.

Results

It is apparent that these compounds have no significant effect on the activity of the alpha-amylase (table 1). The greatest effect (10% decrease by beta-naphthoxyacetic acid at 100 p.p.m.) approximates the limit of experimental error for the comparison of two runs. The low levels of activity at all growth-regulator concentrations used in this particular experiment indicate that the control may have been high. For this tumor tissue these same growth-regulators at 100 p.p.m. cause a growth inhibition of between 90% and 100% and an inhibition

[1] This work was supported in part by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

of respiration amounting to 37% for indoleacetic acid, 10% for 2,4-dichlorophenoxyacetic acid, and 70% for beta-naphthoxyacetic acid (**7**).

Discussion

EYSTER (**3**) reported that indolepropionic acid retarded the action of malt diastase on soluble starch but accelerated the action of the enzyme in the presence of charcoal, presumably by releasing the adsorbed enzyme from the charcoal. The same author later (**4**) mentioned similar action by indolebutyric, indoleacetic, and alpha-naphthaleneacetic acids at concentrations of 25 and 50 p.p.m. and reached the conclusion that the effectiveness of these compounds was due entirely to a pH effect (**4, 5**). However, SMITH *et al.* (**10**) pointed out the lack of pH control in EYSTER's experiments. These workers were unable to duplicate EYSTER's results under controlled conditions. They could find no effect of indoleacetic acid on diastatic activity in the presence or absence of charcoal.

Still other reports indicate effective inhibition of salivary amylase by many plant growth-regulators: indoleacetic, indolebutyric, indolepropionic, alpha-naphthaleneacetic, 2,4-dichlorophenoxyacetic, beta-naphthoxyacetic, and 2,3,5-triiodobenzoic acids (**11, 12**). In these instances the growth-regulators were used at concentrations as low as 100 p.p.m., but no figures were given to indicate what constituted effective inhibition. NEELY *et al.* (**6**) have reported reduced levels of alpha- and beta-amylase activity in the stems and leaves of bean plants treated with 2,4-D.

TABLE 1

EFFECT OF PLANT GROWTH-REGULATORS ON SECRETED α-AMYLASE

GROWTH-REGULATOR	ACTIVITY OF CONTROL*	ACTIVITY (CONTROL = 100%) IN PRESENCE OF GROWTH-REGULATOR† AT		
		1 p.p.m.	10 p.p.m.	100 p.p.m.
2,4-Dichlorophenoxyacetic acid	0.338	96	99	93
	.085	100	97	91
Indoleacetic acid	.090	98	101	104
	.130	100	96	98
Beta-naphthoxyacetic acid	.325	93	98	97
	0.135	94	91	90

* Milligrams of starch hydrolyzed per hour per milliliter of enzyme solution.
† Based on activity of control as 100%.

Summary

1. Plant growth-regulators representing the indole, phenoxy, and naphthoxy types of structure were tested to determine their effect on the activity of an alpha-amylase secreted by virus tumor tissue from the roots of *Rumex acetosa* grown *in vitro*.

2. At concentrations up to and including 100 p.p.m. they had no significant effect on its activity. The greatest effect was within the limit of experimental error.

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LITERATURE CITED

1. BRAKKE, M. K., and NICKELL, L. G. Secretion of α-amylase by *Rumex* virus tumors *in vitro*. Properties and assay. Arch. Biochem. and Biophys. **32**:28–41. 1951.
2. BURKHOLDER, P. R., and NICKELL, L. G. Atypical growth of plants. I. Cultivation of virus tumors of *Rumex* on nutrient agar. BOT. GAZ. **110**:426–437. 1949.
3. EYSTER, H. C. Auxin action. Science **97**:358–359. 1943.
4. ———. Effects of auxins on the action of diastase *in vitro*. Plant Physiol. **21**:68–74. 1946.
5. ———. Amylase inhibition. Science **112**:724. 1950.
6. NEELY, W. B.; BALL, C. D.; HAMNER, C. L.; and SELL, H. M. Effect of 2,4-dichlorophenoxyacetic acid on the alpha- and beta-amylase activity in the stems and leaves of Red Kidney bean plants. Science **111**:118. 1950.
7. NICKELL, L. G. Effect of certain plant hormones and colchicine on the growth and respiration of virus tumor tissue from *Rumex acetosa*. Amer. Jour. Bot. **37**:829–835. 1950.
8. NICKELL, L. G., and BURKHOLDER, P. R. Atypical growth of plants. II. Growth *in vitro* of virus tumors of *Rumex* in relation to temperature, pH, and various sources of nitrogen, carbon, and sulphur. Amer. Jour. Bot. **37**:538–547. 1950.
9. NICKELL, L. G.; GREENFIELD, PHYLLIS; and BURKHOLDER, P. R. Atypical growth of plants. III. Growth responses of virus tumors of *Rumex* to certain nucleic acid components and related compounds. BOT. GAZ. **112**:42–52. 1950.
10. SMITH, F. G.; LANGELAND, W. E.; and STOTZ, E. The effect of indole-3-acetic acid in the diastase charcoal model system. Plant Physiol. **22**:300–307. 1947.
11. VOLKER, J. F. Compounds capable of plant amylase inhibition. Science **112**:61. 1950.
12. VOLKER, J. F., and MURRAY, D. P. Salivary amylase inhibition. Tufts Dental Outlook **21**:3. 1947.

A SAND PRESS METHOD FOR PRESERVATION OF NATURAL COLORS IN HERBARIUM SPECIMENS

Collection and preservation of plant specimens have been essential in the studies of plant pathologists and taxonomists. Not only the preservation of plant materials but retention of their natural colors is desired by botanists, especially by plant pathologists. So far, no method has been available for retaining the natural colors of herbarium specimens. The various presses used for preparation of such specimens have been unsatisfactory because: (*a*) a single worker making large-scale collections finds repeated changes of blotting paper to hasten drying of the pressed specimens laborious and time-consuming; (*b*) thicker structures, such as the sori and galls of rusts, smuts, etc., are crushed; (*c*) colors are rarely maintained.

After unsatisfactory experience with this traditional method, the writer has recently successfully evolved a labor-saving, cheap way to prepare herbarium specimens in natural colors. This method uses layers of clean, dry, sand of medium texture to press the specimens and, at the same time, through porosity of the sand mass, provides ample aeration which facilitates rapid removal of moisture from the specimens. A level layer of sand 1 inch thick is spread on a platform, and an absorptive paper is laid over the sand. The specimens are placed on it without overlapping and then covered by a similar sheet of paper. Over this is spread another layer of sand 1 inch thick. This process is repeated, alternating layers of sand with plant specimens until a stack 10 inches high is made, thus pressing five layers of specimens. The intervening layers of sand are leveled each time.

The stack is kept exposed, preferably under a powerful fan or facing a constant wind current. Specimens dry in about 3–5 days depending upon the factors that affect rapid removal of moisture, such as humidity, wind velocity, breadth and height of the stack, thickness of the sand layers, etc. The specimens may be taken out after this period or may be left indefinitely. To remove dried material the sand layer is carefully pushed aside, the top sheet of paper below it is lifted from one side, and the pressed specimens then lie exposed on the other sheet. They are dry, flat, and retain their natural colors to a remarkable extent. Since the sand mass tends to distribute pressure uniformly, morbid and natural prominences on the plants are not crushed. The unhindered rapid removal of moisture from the fresh specimens through the porous sand apparently aids preservation of the plant pig-

ments, through limiting the destructive activities of molds and bacteria, and the autolytic processes of the living tissues themselves which affect the pigment system. The physiological significance of this case of pigment preservation, however, has yet to be properly investigated.

These specimens with natural colors keep well indefinitely if not carelessly exposed to too much moisture. Ordinarily specimens in the paper packets may be kept in bigger packets of paraffin-impregnated, cheap, white paper (labeled before paraffin impregnation) in steel cabinets.

In pressing large-scale collections of specimens in the laboratory, the writer has used simple equipment of moderate cost (fig. 1) consisting of a wooden rack 4 feet × 2 feet × 6 feet in size with four planks set horizontally at a distance of 1½ feet above each other. On each plank a pile of sand 3¾ feet × 1½ feet × 10 inches high pressing five layers of specimens is made as described. As specimens are taken out sand collects at the bottom of the rack and is reused for subsequent pressings.

The following precautions are essential. (*a*) Individual sand layers should not be so thick as to delay drying. Too thin layers, however, lead to shriveling of specimens owing to insufficient weight, especially when conditions favor too rapid drying. (*b*) The stack may be any convenient length but should not be more than 10 inches high and 1½ feet wide for proper aeration of the materials. (*c*) Low humidity and high wind conditions give best results.

This "sand press" method is recommended for general preparation of herbarium specimens because: (*a*) the natural plant colors are retained; (*b*) gall and sorus structures of phytopathological specimens are not crushed; and (*c*) since drying and pressing processes are combined in one operation, it is cheap, simply, and labor-saving. Technical and commercial possibilities of the method are under detailed investigation.

The writer is indebted to a forgotten village girl whose methods, observed by him a decade ago, led to the present findings. He is highly grateful to AKSHAIBER LAL, Head of the Section of Plant Pathology and Mycology, College of Agriculture, for his kind encouragement and guidance.—B. T. LINGAPPA, *Section of Plant Pathology and Mycology, College of Agriculture, Benares Hindu University.*

FIG. 1.—Wooden rack with four platforms supporting sand layers alternating with herbarium specimens in blotters. Oscillating fan in position in back.

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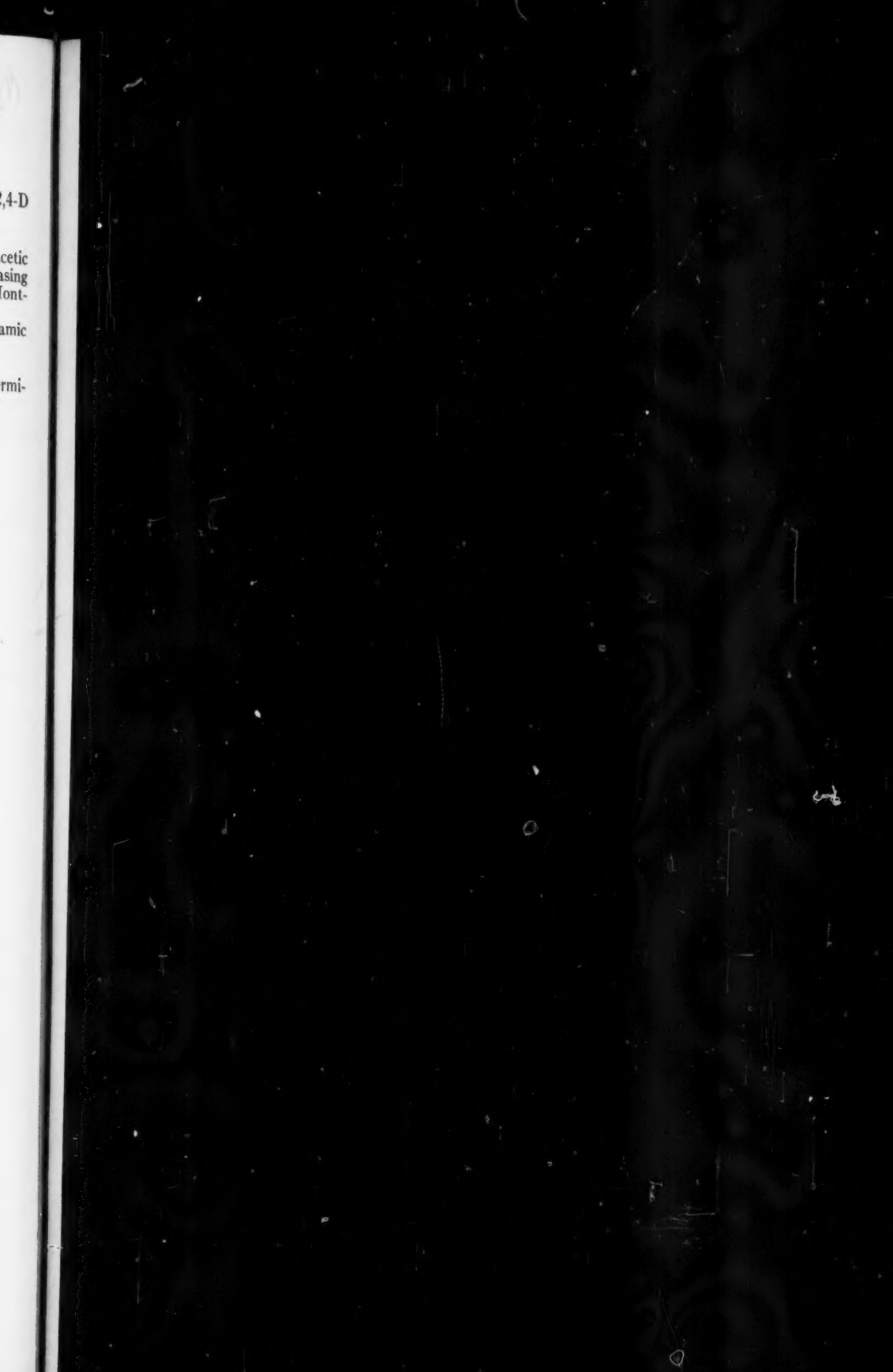
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